Technical Bulletin 106 July 1935

University of Minnesota
Agricultural Experiment Station

The Pathogenicity and Genetics of Gibberella Saubinetii (Mont.) Sacc.

Carl J. Eide
Division of Plant Pathology and Botany

UNIVERSITY FARM, ST. PAUL

University of Minnesota
Agricultural Experiment Station

The Pathogenicity and Genetics of Gibberella Saubinetii (Mont.) Sacc.

Carl J. Eide
Division of Plant Pathology and Botany

UNIVERSITY FARM, ST. PAUL

CONTENTS

THE PATHOGENICITY AND GENETICS OF *GIBBERELLA SAUBINETII* (MONT.) SACC.[1]

CARL J. EIDE[2]

INTRODUCTION

The complexity and variability of the genus *Fusarium* offers one of the chief problems confronting mycologists and plant pathologists. The large number of destructive diseases caused and the wide range of host plants attacked by these fungi make necessary a means whereby they may be accurately identified. Again, the nature of the diseases produced is such that the production of resistant varieties of host plants is the most desirable and, in many cases, the only possible means of control. The attainment of these ends is made extremely difficult by the large number of taxonomic entities comprised in the genus and their extreme variability in pure culture. Naturally, the attention of many investigators has been drawn to these problems. Among the foremost are Wollenweber (57, 58, 59), Sherbakoff (44), and Coons and Strong (15), who studied the taxonomic phase primarily, and Brown (7, 8) and Leonian (33, 34), who investigated the variation in the genus and its bearing on an adequate system of classification. These investigators worked primarily with entities for which no sexual stage is known.

In view of this fact it was thought that the problem might be further elucidated by a study of a species of *Fusarium* which normally produces a perfect stage. This has been suggested by Snyder (47). The possible advantages of such a procedure are several. In the first place the presence of the sexual fruiting bodies and spores makes possible a more accurate specific determination. It would be possible, by using such a species, to determine the extent of variability as it occurs in nature, without the long cultural procedure necessary to determine a species by the characteristics of the asexual stage, during which process variations may, and usually do, occur. The stability of the sexual stage might be studied, both as regards the occurrence of the sexual fruiting bodies in culture and the stability of cultural variants when reproduced

[1] Presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree granted June, 1934.

[2] The author wishes to express his sincere thanks to Prof. E. C. Stakman, under whose direction these investigations were made. He also wishes to acknowledge his deep indebtedness to Dr. E. L. LeClerg, of the U. S. Department of Agriculture, for assistance with the statistical phases of the problem and to Dr. J. J. Christensen and Dr. Louise Dosdall for their willing and competent advice and criticism.

by sexual spores. Finally, if the fungus should prove heterothallic, a genetic study might be made of the segregation and recombination of the variant types.

Gibberella saubinetii (Mont.) Sacc., the perfect stage of *Fusarium graminearum* Schwabe, is a common pathogen on cereals and corn in the United States and other parts of the world. In Minnesota it produces head blight or scab of wheat and barley, frequently causing heavy reductions in yield and rendering the barley unfit to feed to swine. It also produces rots of the roots, stalks, and ears of corn as well as seedling blight of corn and small grains.

The perithecia of *G. saubinetii* are produced abundantly on corn stubble in Minnesota. Spores from these perithecia are principally responsible for primary infection of wheat and barley if these crops are planted on the same field without first plowing under the corn stubble.

GENERAL HISTORICAL REVIEW

The habitat of *G. saubinetii* includes a wide range of plants. Atanasoff (4) lists 36 genera in 15 different families upon which it has been found. Its geographic distribution includes North and South America, Europe, Asia, Australia, and Africa. The pathogen causes a head blight and seedling blight of wheat, emmer, rye, oats, spelt, barley and rice. It also produces root and shank rot and seedling blight of corn. Kasai (30) reports a node rot of rice caused by *G. saubinetii,* while Wollenweber (57) found that it was capable of causing a rot of sweet potatoes.

Extensive reviews of the literature dealing with Fusarial blight of cereals have been given by Atanasoff (4) and by McInnes and Fogelman (35). Atanasoff (4) lists eleven species and varieties of *Fusarium* which will attack cereals. Of these, eight may produce head blight, *G. saubinetii, Calonectria graminicola* (Berk. et Brm.) Wr., *F. culmorum* (W. G. Sm.) Sacc., and *F. avenaceum* (F.) Sacc. being the most common. Their geographical distribution apparently determines to a large extent their importance in this respect. Thus, *G. saubinetii* is common in the United States, Holland, Bulgaria, and southern Russia; *C. graminicola* in Germany and the Scandinavian countries; *F. avenaceum* in northern Russia; and *F. culmorum* in Holland, Germany, Sweden, France, and Oregon, U.S.A. Dickson, Johann, and Wineland (20) report that 94 to 98 per cent of the scab in the spring wheat area of the United States is due to *G. saubinetii.*

Fusarial head blight was first reported in 1884 by Smith (46) in England. In 1890 Woronin (60), according to Atanasoff (4), reported

G. saubinetii as associated with grain producing "inebriating bread." Bread possessing this intoxicating quality is now known to be produced from scab-infected grain. Sorokin (48), in 1891, observed *G. saubinetii* on blighted wheat heads in the Usurian provinces. Chester (11) and Weed (55) described head blight of wheat in the United States in 1890, attributing it to *F. culmorum*. Their drawings, however, indicate that the fungus was *G. saubinetii*. Selby (42), in 1898, considered blighting of wheat heads to be due to *F. roseum,* which he thought was the imperfect stage of *G. saubinetii.*

Selby (42) was unable to produce head blight by artificial inoculations with conidia of *Fusarium,* but Selby and Manns (43) demonstrated, in 1909, by spraying plots of wheat and oats with conidial suspensions, that *G. saubinetii* was able to produce the disease. By cross inoculations they showed that the same organism was responsible for head blight on wheat, oats, barley, emmer, and spelt.

The numerous articles on occurrence of head blight have been well summarized by Atanasoff (4), who described fully the seedling blight and foot rot stages of the disease, as well as the head blight. He also studied factors affecting infection, sources of natural infection, and methods of overwintering, and described in some detail the life history of *G. saubinetii,* as well as that of other *Fusaria* which may attack cereals.

Varietal differences in susceptibility to scab have been demonstrated by Christensen, Stakman, and Immer (14) and others, while Tu (54) showed that *F. graminearum,* as well as certain other species of *Fusarium,* comprise strains which differ decidedly in their pathogenicity on heads of cereals.

Selby and Manns (43) found that *G. saubinetii* infesting wheat seeds would cause the death of the wheat seedlings. This phase of the problem has also been described fully by Atanasoff (4), while Dickson (19) has investigated the relations of temperature to seedling blight caused by *G. saubinetii.* Henry (25), who tested the pathogenicity of numerous fungi isolated from wheat seed, states that *G. saubinetii* was most virulent of all the fungi he studied, as measured by its capacity to blight wheat seedlings in the greenhouse. Greaney and Bailey (23) reported that four cultures of *Fusarium,* tentatively identified as *G. saubinetii,* differed widely in pathogenicity on wheat seedlings.

Gibberella saubinetii is recognized as one of a number of fungi that may cause root, stalk, and ear rots of corn. This has been demonstrated by Hoffer and Holbert (26) and by Manns and Phillips (36), who state that *G. saubinetii* was the most active seedling parasite studied. The relationship of temperature and nature of resistance have been

studied by Dickson (19) and his coworkers, while McIndoe (37) and Hayes, Johnson, and Stakman (24) have studied the inheritance of resistance in corn to seedling blight caused by this fungus.

Hoffer, Johnson, and Atanasoff (27) pointed out in 1918 that wheat sown on unplowed corn land was liable to heavy attacks of head blight, thus showing the intimate practical relationship of these diseases caused by the same fungus. The importance of this phenomenon has been further emphasized by Koehler, Dickson, and Holbert (31).

The above review of the more important developments in the knowledge of the pathogenicity of *G. saubinetii* indicates what course future research on this organism must take. One of the most important—the investigation of the variability, stability and genetics of the pathogen—is the subject of the following researches. Other pertinent literature bearing on these and related subjects will be found cited in the accounts of investigations which follow.

OBJECTS OF THE INVESTIGATION

The specific objects of the investigation were, first, to determine the range of variability of *G. saubinetii* as it occurs in nature; second, to observe variations which might occur while the fungus was growing in culture, chiefly to compare it in this respect with the behavior of other *Fusaria* as reported by other workers; third, to study factors affecting the appearance of the perfect stage; fourth, to determine whether the fungus is homothallic or heterothallic, and to study the segregation and recombination of cultural characters if it should prove heterothallic; and, fifth, to study the pathogenicity of different lines.

EXPERIMENTAL WORK

Sources of Cultures

Cultures of *Gibberella saubinetii* used in the following studies were, with one exception, started from single ascospores obtained from perithecia on old corn stubble in grain fields in Minnesota. In addition, perithecial material was furnished through the kindness of Mr. A. L. Smith of the Bureau of Agricultural Economics, U. S. Department of Agriculture, who collected it in the summer of 1931 in Minnesota, Wisconsin, and Iowa. A culture also was used which had been grown on potato-dextrose agar for a number of years in the Division of Plant Pathology and Botany at the University of Minnesota. This culture was used by Tu (54) under the designation *Fusarium graminearum* form 1 and was originally procured from Prof. L. R. Jones of Wisconsin.

Table 1 shows the sources of the various collections. The collection numbers are used to designate the various cultures throughout the entire experimental work.

Table 1

Sources of Collections of *Gibberella saubinetii*

Collection number	Source of collection	Year	Location
1	Corn stubble, wheat field	1932	Cokato, Minn.
2	do	do	Atwater, Minn.
4	do	do	Willmar, Minn.
7	do	do	Haydonville, Minn.
9	Culture used by Dr. Tu (56)		Originally obtained from Wisconsin
12	Corn stubble, wheat field	1932	Madison, Minn.
14	do	do	Madison, Minn.
16	do	do	Clarkfield, Minn.
17	do	do	Clarkfield, Minn.
18	Corn stubble, barley field	do	Marshall, Minn.
26	do	do	Avoca, Minn.
27	do	do	Fulda, Minn.
28	Corn stubble, wheat field	do	Fairmont, Minn.
29	Corn stubble, barley field	do	Fairmont, Minn.
30	do	do	Fairmont, Minn.
31	do	do	Fairmont, Minn.
32	do	do	Fairmont, Minn.
34	do	do	Guckeen, Minn.
35	Corn stubble, wheat field	do	Blue Earth, Minn.
36	Corn stubble, barley field	do	Walter, Minn.
38*	do	1931	Emmetsburg, Ia.
39	Corn stubble, wheat field	1932	Hollandale, Minn.
41	Corn stubble, barley field	1933	Mendota, Minn.
42	Corn stubble, wheat field	do	Mendota, Minn.
43	Corn stubble, barley field	do	Rosemount, Minn.

* Furnished by Mr. A. L. Smith.

Materials and Methods

Except where special media were used, the principal substrata used in the cultural work were potato-dextrose agar and the synthetic agar medium described by Coons and Strong (15)[3].

Single ascospores and conidia were isolated with a glass needle operated with a Zeiss micromanipulator, using the method described by Dickinson (18). The single spores were transferred by means of the needle to drops of agar medium on cover slips, where they were allowed to germinate. The hyphae were allowed to grow for a day or two and were then transferred to agar slants, or, in some cases, directly to the medium in Erlenmeyer flasks or petri dishes.

Hyphae were cut from individual cells of germinating conidia and ascospores by the following method. Pieces were broken from a new

[3] The potato-dextrose agar consisted of: peeled potatoes, 400 grams; dextrose, 10 grams; agar, 17 grams; and distilled water, 1 liter. The Coons' synthetic medium consisted of: sucrose, 7.20 grams; dextrose, 3.60 grams; $MgSO_4$, 1.23 grams; KH_2PO_4, 2.72 grams; KNO_3, 2.02 grams; agar, 17 grams; and distilled water, 1 liter.

safety razor blade and a piece with a cutting edge 0.5 mm. or less in length was soldered to the point of a sewing needle in such a manner that the cutting edge was approximately parallel to the long axis of the needle. The needle was then inserted into a glass tube drawn out so the small end would just receive the needle. It was firmly fastened in this position with sealing wax previously drawn into the tube. This cutting tool was then operated with a Zeiss micromanipulator.

In practice, the ascospore or conidium was allowed to germinate on an agar drop until the germ tubes were approximately 200μ long. A drop of weak malt solution was placed on the agar drops to prevent the growth of the hyphae into the agar. When germ tubes had attained the proper length, the malt solution was removed with a bit of sterile filter paper, and the agar drop was allowed to dry in a sterile petri dish until no liquid remained on the surface. The cover slip bearing the agar drop was then placed over the cell used in isolating single spores. The cutting blade was elevated until the edge touched the hypha which was to be removed, the cover slip being turned until the hypha was at right angles to the blade. The blade was then drawn across the hypha, cutting it off. Single hyphae, thus severed, were drawn to one side of the drop and transferred, with a piece of the agar, to a fresh drop. Here the development could be watched until the hyphae were transferred to agar slants. It was found that if the hyphae were transferred without the agar to a new drop, as spores are, they almost never survived.

The methods used in pathogenicity studies are described in the section on pathogenicity.

CULTURAL TYPES OF FIELD ISOLATES

To determine the cultural types of *Gibberella saubinetii* found in nature, the following experiment was made. Five single ascospores, taken at random from several perithecia of each of 25 collections, were isolated with the micromanipulator. These were placed on slants of potato-dextrose agar and, after a few days, were transferred to petri dishes containing Coons' synthetic agar medium. They were allowed to grow at room temperature. Six of the collections were found to be *Gibberella moniliformis* Sheldon. Cultures from the remaining nineteen collections were examined 16 days after being transferred. It was at once apparent that most of them, while somewhat different in shade of color, were of essentially the same type and grew at the same rate. It was noted that, with the two exceptions mentioned below, each of the five isolates from a single collection were of the same shade of color when viewed through the bottom of the petri dish. The color of the whole group, exclusive of the three exceptional types mentioned below,

ranged from deep Eugenia red[4] to pale pink. All shades between the two extremes were represented, making it impossible to group the isolates on this basis. When they were arranged in a series according to color shade, the collections fell in the order given in Table 2. (See Plates I and II, OA29-II and OA39-V.)

Table 2

Order of Collections of *Gibberella saubinetii* When Arranged According to Color Intensity of Submerged Mycelial Growth of Isolates from the Collections; Darkest Culture First

First experiment:	39, 36, 34, 27, 14, 35, 18, 17, 12, 7, 1, 2, 4, 32, 28, 16, 30, 26, 29
Second experiment:	34, 39, 14, 27, 36, 41, 43, 28, 42, 32, 12, 17, 4, 16, 2, 31, 18, 26, 7, 35, 30, 29

Slight differences were noted in the character and color of the aerial mycelium. These are presented in Table 3. It can be seen that there was no correlation between the shade of color of the culture as seen through the bottom of the dish and the type of mycelial growth.

Table 3

Character of Aerial Mycelial Growth of Isolates from Field Collections. First Experiment Only

Mycelial type	Collections represented
Very light pink, cottony, nearly filling dish	1, 2, 16, 17, 28, 30
Darker pink than above, cottony, filling dish in most instances	4, 19, 18, 27, 39
Pink with yellowish tinge, cottony, filling dish but later shrinking somewhat	7, 12, 26, 29, 32, 34, 35, 36

Seven lines of the above 95 cultures were exceptional in their appearance. One, designated line OA39-III[5], (See Plates I and II, OA39-III) developed practically no aerial mycelium but later formed perithecia in abundance. It was orange pink, with a purplish border. Lines OA31-I to OA31-V, inclusive (see Plates I and II, OA31-I), grew much more slowly than the predominant type and produced very little aerial mycelium. The color was cinnabar red and the border very irregular in outline. A third exceptional type was OA12-V (see

[4] Colors given are those described in Ridgway's Color Standard (41). While it is extremely difficult to match the color of these fungi with these standards, the nearest possible shade is given.

[5] In the system of cultural nomenclature here used, OA indicates an ascospore picked up at random. If a series is taken from a single ascus, the letter A is used. The Arabic numeral following indicates the collection number and the roman numeral the ascospore. If eight ascospores are isolated from a single ascus, an Arabic numeral, designating the ascus, is inserted between the collection number and the ascospore number. Thus OA39-III indicates the third ascospore picked at random from collection 39. A36-1-V would designate the fifth ascospore from ascus number 1 of collection 36. Cultures arising as sectors are given the numerical designation of the parent with the addition of the abbreviation sec.

Plates I and II, OA12-V). This type was found often as a variant in the studies on variation. It was the mottled type designated as C in Table 5.

Whether or not these exceptional types may be accepted as naturally occurring physiologic forms of the fungus, is hard to say. Since all five isolates of collection 31 were of the single type in the first experiment, one might be justified in saying in this instance they were. On the other hand, when the experiment was repeated collection 31 yielded cultures that were all of the normal type.

Lines OA39-III and OA12-V might justifiably be regarded as variants which appeared very early in the cultural life of the isolate, especially since similar types have been observed as variants in old cultures. In fact, type C, represented by OA12-V, arose from a single ascospore from a perithecium developing in pure culture. The parent culture had been repeatedly propagated from ascospores, and seven other ascospores isolated at the same time from the same ascus were true to the parental type.

The isolation of naturally occurring ascospores was repeated in the way described above, with the addition of three new collections. The results were essentially the same, except that the collections did not fall into the same order when arranged according to shade of color. It is also true that no exceptional types appeared among this group of isolates. The five cultures from each collection did not agree quite so well in shade of color as they did in the first experiment. This was due, no doubt, to the fact that they were unavoidably held in culture on slants longer before being transferred to plates. Nevertheless, they were sufficiently alike to be grouped together. The order of collections arranged according to shade is given in Table 2.

In addition to the above experiments, the eight ascospores were isolated from each of fifteen different asci, representing seven collections. It was here proposed to study cultural differences that might occur within the eight lines from any individual ascus. However, they were found to be identical in every instance, both on potato-dextrose agar and on Coons' agar.

Most of these isolates were of the darker type described above, although collection 42 was an exception. When first isolated and transferred to slants of potato-dextrose agar, twenty-four isolates from three asci of this collection were identical with twenty-four from collection 43. When transferred to Coons' medium in plates, however, they produced colonies with deeply lobed margins, as contrasted with the almost perfect circles formed by the other isolates. The abundant aerial mycelium was also lacking, the mycelial mat being appressed and

having a wet surface. They were Eugenia red, with a strong yellow tinge. As they grew older, aerial mycelium developed, giving the colonies an appearance more like that of the predominant type.

Thus, of a total of 325 isolates from naturally occurring ascospores, 318 were of the same cultural type, differing only in color intensity. Twenty-four additional isolates were distinguished from the predominant type by differential characters on Coons' medium. It is possible that a greater number of media would have resulted in further differentiation.

VARIATION

The production of cultural variants, such as have been observed in numerous groups of fungi, is common in the genus *Fusarium*. Brown (7) first discussed variation in this genus, referring to the variant types as saltations. He (8) points out that Appel and Wollenweber (2) had previously described phenomena which probably indicated variations of the same type. Leonian (33, 34) describes and discusses in detail numerous variants arising in several species of *Fusarium*. Similar variations in this genus have been reported by Tu (54), Mitter (38), and others. On the other hand, Coons and Strong (15) state that they did not observe variations in their investigations of a large number of species. Leonian (34), however, claims he can detect sectors in photographs of cultures appearing in the paper by Coons and Strong.

The behavior of *Gibberella saubinetii* in culture was substantially the same as that of other species of *Fusarium*. Single ascospore lines carried on Coons' agar in tubes were observed to change radically in cultural appearance. To better observe the nature and frequency of these changes, forty lines arising from ascospores originating in five asci were grown on Coons' agar in flasks for seven cultural generations. Later, another series of eight ascospores from one ascus were added and grown for three cultural generations, both on Coons' agar and on potato-dextrose agar. Some of the former lines had been carried in culture for a time and had already changed in cultural appearance, while others had been recently isolated from naturally occurring perithecia and were of the normal cultural type when the experiment was started.

Records were kept of the cultural type at the beginning and end of the experiment and also of the approximate number of sectors that appeared in each strain. These observations are recorded in Table 4.

No attempt was made to isolate the sectors except in a few cases when it was desired to study their pathogenicity. The variant types

Table 4

Variations and Cultural Behavior of Single Ascospore Lines of *Gibberella saubinetii*

Line	Initial cultural type*	Final cultural type	Number of sectors observed	Remarks on cultural behavior
Lines grown seven cultural generations on Coons' synthetic agar medium				
A38-1-I	I	I	0	Cultural type very constant
A38-1-II	H	H	0	Variable; center occasionally being cream or concentric cream and red rings
A38-1-III	C	C	0	Cultural type constant
A38-1-IV	D	D	0	Cultural type constant
A38-1-V	D	D	0	Tendency to mottling shown in some cultures
A38-1-VI	C	C	0	Two cultures had a slight reddish border
A38-1-VII	..	..	..	Contaminated with bacteria
A38-1-VIII	A	A	2	One culture was very light but became normal later
A39-1-I	A	B	7+-	Small sectors very numerous
A39-1-II	A	E	10+	Sectors numerous. Changed type in fifth generation
A39-1-III	A	B	15	
A39-1-IV	A	A	2	Slightly mottled in two cultures
A39-1-V	A	A	3	Slightly mottled in two cultures
A39-1-VI	A	A	6	Fairly constant
A39-1-VII	A	A	8	Fairly constant
A39-1-VIII	A	D	3	Changed type in sixth generation
A36-1-I	A	A	4	Fairly constant
A36-1-II	A	B	7	
A36-1-III	A	A	Numerous	Unchanged except aerial mycelium became white
A36-1-IV	A	A	5	Very irregular, sometimes growing very slowly; finally normal except for pink aerial mycelium
A36-1-V	A	A	3	Tendency to mottling at times; finally normal except for pink aerial mycelium
A36-1-VI	C	C	0	Fairly constant
A36-1-VII	A	C	0	Changed type in sixth generation
A36-1-VIII	C	C	0	Fairly constant
A35-1-I	D	D	0	Fairly constant
A35-1-II	A	D	2	Changed type in third generation. Fairly constant since
A35-1-III	C	C	5	Fairly constant
A35-1-IV	E	F	2+	Numerous patch variants
A35-1-V	C	G	0	Irregular; red with large light patches at times
A35-1-VI	A	C	1	Changed type in fifth generation
A35-1-VII	A	B	27	Behavior very irregular
A35-1-VIII	A	B	6	Normal except for rather definite sectors
A12-1-I	A	B	2	Mottled or cream in some cultures
A12-1-II	A	B	5	Changed type in fifth generation
A12-1-III	G	..	..	Contaminated with bacteria
A12-1-IV	A	F	6	Behavior irregular; changed type in seventh generation
A12-1-V	A	D	9	Behavior irregular; changed type in seventh generation
A12-1-VI	A	B	3	Irregular; appearing slightly mottled at times
A12-1-VII	A	E	3	Irregular; changed type in sixth generation
A12-1-VIII	A	E	1	Changed type in fourth generation
Lines grown three cultural generations on Coons' agar				
A43-4-I	A	A	0	Fairly constant
A43-4-II	A	G	1	Changed type in second generation
A43-4-III	A	B	Several	Sectored very freely
A43-4-IV	A	A	1	One flask type C in third generation
A43-4-V	A	A	1	Fairly constant
A43-4-VI	A	A	4	Fairly constant
A43-4-VII	A	A	1	Constant
A43-4-VIII	A	A	0	Tendency to mottling shown in first generation
Lines grown three cultural generations on potato-dextrose agar				
A43-4-I	A	A	5	Fairly constant
A43-4-II	A	A	0	Fairly constant
A43-4-III	A	A	0	Fairly constant
A43-4-IV	A	A	1	Fairly constant
A43-4-V	A	A	0	Fairly constant
A43-4-VI	A	A	0	Fairly constant
A43-4-VII	A	A	0	Fairly constant
A43-4-VIII	A	A	0	Fairly constant

* These cultural types are described on pp. 13 and 15.

listed in Table 5 first appeared, not as sectors, but as entire colonies of the variant types, following the transfer of the cultures from one set of flasks to another. Effort was made to transfer the parent type from a flask in which sectors occurred, but, even so, many strains changed type, probably because of the transfer of small, unobserved variants in the parent culture. The parent types were, of course, lost in this procedure. It is surprising that, with this procedure, the variant type often appeared in both flasks of a duplicate set. Pictures of eight sister ascospore isolates, showing variation, are shown on Plates III and IV.

Fine distinctions between individual cultures were not made, as it was found that minor variations in appearance might occur from one cultural generation to the next and even between duplicate cultures transferred at the same time. However, the variants were usually sufficiently characteristic to be easily placed in one of a few groups, which can be described in relatively simple terms. A brief description of these groups or types, which are designated by capital letters in Tables 4 and 5, is given below.

Type A. Referred to in this paper as the "normal" type; characterized by abundant, cottony, aerial mycelium, red, often with a tinge of yellow. The bottoms of the cultures were pink to deep Eugenia red.

Type B. The normal type producing sectors. This might be considered the same as A but is separated in Table 5 to show the number of strains which were producing sectors in the final cultures of the experiment.

Type C. Characterized by white or pink aerial mycelium, which soon collapsed and adhered to the surface of the colony, appearing wet. The bottom of the colony was characteristically mottled in red and cream.

Type D. Aerial mycelium white; bottom of culture cream, with a border of purplish red to pink about one centimeter wide. Fluctuation in this type was very common.

Type E. Aerial mycelium white; bottom of culture cream.

Type F. Aerial mycelium white; bottom of culture pink.

Type G. Aerial mycelium white or pinkish and persisting, as contrasted with Type C; bottom finely mottled similar to C. This type was somewhat similar to C but was sufficiently different to be recognized easily and seemed fairly distinct from one cultural generation to the next.

Type H. Aerial mycelium white; bottom of culture mottled as in Type C, but with a distinct red or purplish red border.

Type I. No aerial mycelium produced; slimy masses of conidia formed, producing a colony described as pionnotes by Wollenweber (57).

Table 5

Distribution of Variant Types in Single Ascospore Lines of *Gibberella saubinetii* After Being Grown for Seven Cultural Generations on Coons' Synthetic Agar Medium*

"Normal"; red to Eugenia red	"Normal"; sectoring	Mottled	Cream; pink or red border	Cream	Pink	Red; slightly mottled	Mottled; red border	Buff; no aerial mycelium	Purple	Perithecial
A†	B	C	D	E	F	G	H	I	J	K
A38-1-VIII	A39-1-I	A38-1-III	A38-1-IV	A39-1-II	A35-1-IV	A35-1-V	A38-1-II	A38-1-I	A43-4-IIIsec	A43-4-I-I
A39-1-IV	A39-1-III	A38-1-VI	A38-1-V	A12-1-VII	A12-1-IV	A43-4-II				A43-4-I-II
A39-1-V	A36-1-II	A36-1-VI	A39-1-VIII	A12-1-VIII		A38-1-VIIIsec				A36-1-Vsec
A39-1-VI	A35-1-VII	A36-1-VII	A35-1-I							A36-1-VIIIsec
A39-1-VII	A35-1-VIII	A36-1-VIII	A35-1-II							OA39-III
A36-1-I	A12-1-I	A35-1-III	A12-1-V							
A36-1-III	A12-1-II	A35-1-VI								A43-4-VIsec
A36-1-IV	A12-1-VI									
A36-1-V	A43-4-III									
A43-4-I										
A43-4-IV										
A43-4-V										
A43-4-VI										
A43-4-VII										
A43-4-VIII										

* Lines A38-1-I to VIII, inclusive, were grown for several cultural generations on Coons' agar previous to the time these records were started. Lines A43-4-I to VIII, inclusive, were grown for only three generations.

† The letters indicate the cultural types, as described on pp. 13 and 15.

Type J. This type, represented by a single line, arose from a single conidial isolation made from line A43-4-III. When young, this line produced a rather tough mycelial mat which was salmon-buff. Later it turned a deep purple, due to the formation of thick, heavy-walled hyphae, similar in appearance to the walls of the perithecia. At this stage the colony, when on Coons' agar, had a metallic glint, and was covered by a very scant growth of white aerial hyphae. On potato-dextrose agar the appearance was somewhat the same except that a small area, 1.5 centimeters in diameter, retained the salmon-buff color. The growth of aerial hyphae was also slightly more abundant.

Type K. This type, altho consisting of a number of recognizable cultural individuals, will be for the present designated merely as the "perithecial" type, since the lines included arose as sectors producing perithecia or were isolated from ascospores produced in culture. Further description of these cultures will be found in the section on perithecial production.

It can be seen from examination of Tables 4 and 5 that cultural variation is common in *Gibberella saubinetii*. The variants, once established, apparently remain true to type on the medium used. This was true when they were propagated from single conidia. It was further shown by extensive isolations of hyphae from single cells of conidia and ascospores that the perithecial types remained true to type when propagated from single cells.

No distinct cases of reversion to the original type were observed. Altho there was some irregularity in appearance in some of the lines (see Table 4), these could not be considered cases of reversion, because no definite variant type seemed to become established. This observation does not agree with that of Leonian (34), who observed cases of reversion of cultural type in *Gibberella moniliformis*.

It will be noted that there are relatively few variant types. This substantiates in part the view of Leonian (33), who maintains that the phenomenon of variation is a process whereby the fungus traces the different phases in its cyclogeny, and that the number of types represented is limited by the extent of its cyclogenic phases. The correctness of this viewpoint is open to question. It seems significant, however, that a single type, such as the mottled type C should occur as often as it does. It is possible, of course, that other types arise just as frequently but are obscured by the parent colony because of their slower growth. This might easily have been true of line A43-4-IIIsec, which was started from a single conidium isolated from what appeared to be a uniform parent colony.

OBSERVATIONS ON THE FORMATION OF PERITHECIA

The perithecia of *Gibberella saubinetii* develop abundantly on diseased plant parts, especially old corn stubble, but they are formed only sporadically in culture. Appel and Wollenweber (2) state that the perfect stage of the fungus will develop on sterilized plant stems. Atanasoff (3) found the same to be true, and he emphasized the fact that the cultures must be in what Appel and Wollenweber refer to as the "normal" cultural condition. Atanasoff states further that an abundance of moisture is necessary, and he devised a special culture tube to provide it. He also found that an unidentified bacterium stimulated perithecial formation.

The observations of several investigators indicate that different strains of *G. saubinetii* differ in their ability to produce perithecia. Adams (1) noted that a culture isolated from corn had a tendency to form the perfect stage in culture, while one from wheat did not.

Kasai (30) isolated several conidia of *G. saubinetii* from rice grains and several ascospores from material collected in the field. He found that two of the conidial cultures produced perithecia after seven and nine months, respectively, the first on rice meal agar and the second on boiled potato slices. Kasai isolated ascospores from the above cultures, but does not state whether the resulting cultures also formed perithecia.

Hynes (28) isolated the fungus from perithecia occurring on stubble of oats in Australia. He grew it on a variety of media and followed the method of Atanasoff for inducing perithecial formation, but without success. A culture secured from Prof. L. R. Jones of Wisconsin, however, produced perithecia abundantly under virtually all conditions. This is possibly the same strain (culture 9) which was used in the present studies.

Bennett (5) isolated *G. saubinetii* from wheat and barley grains. He found that rudimentary perithecia were produced on wheat, oats, glycerine, and potato agar, but found no ascospores. Bennett was able to induce the development of ascospores by growing the cultures on sterilized wheat and then placing them in soil in pots. Two types of cultures developed from ascospores isolated from perithecia which were developed under different conditions of substratum, temperature, and moisture. The first produced abundant mycelium and a few conidia. The second formed perithecia on the four agar media previously mentioned. Bennett does not state whether or not mature ascospores developed in these perithecia.

In the present study, only one of 325 single ascospore isolates pro-

duced perithecia when first isolated. This line, OA39-III, has never produced ascospores. Line 9, secured some years ago from Prof. L. R. Jones of Wisconsin, produced perithecia and ascospores under practically all cultural conditions to which it was subjected. During the course of the investigations five other lines acquired the ability to produce perithecia in culture. This phenomenon will be described later.

Various preliminary experiments were made in an effort to induce formation of perithecia in the sterile cultures. These are listed below and, except where noted, were without the desired results.

1. Eight ascospore isolates from a single ascus were mated in all possible combinations on malt agar in petri dishes. After being held three weeks at room temperature (21-25° C.), they were held at 10° C. for four days and again returned to the laboratory. This experiment was repeated at various times with different cultures on potato-dextrose agar, oatmeal agar, and Coons' agar.

2. Fine quartz sand was sterilized in petri dishes and thoroughly wet with a nutrient medium consisting of the salts and sugars used in Coons' agar medium. To half of the plates was added an extract of yeast made by grinding five yeast cakes in a mortar with fine sand and water and filtering through a Berkfeld filter. These media were inoculated with eight single ascospore isolates from one ascus mated in all possible combinations. They were kept moist with sterile distilled water for several months but no perithecia appeared.

3. Twenty-nine cultures were grown singly on Richard's[6] agar in petri dishes at room temperature.

4. Fifty-one cultures were grown singly on potato plugs in test tubes at room temperature.

5. Eight single ascospore isolates from a single ascus were grown on Coons' and Leonian's[7] agar. After nineteen days at laboratory temperature the plates were placed in an incubator at room temperature and treated on three successive days with ethylene gas at a concentration of 1-1000.

6. Five single ascospore isolates were grown on malt agar and on malt agar plus one gram of animal charcoal per 250 cc. of the medium. One of these lines, A36-1-Vsec, was then producing perithecia on Coons' agar and on potato-dextrose agar. This culture formed perithecia on the media used in this experiment also but in smaller numbers than on Coons' or potato-dextrose agar.

[6] The composition of Richard's agar was: Cane sugar, 50 grams; KNO_3, 10 grams; KH_2PO_4, 5 grams; $MgSO_4$, 2.5 grams; $FeSO_4$, a trace; agar, 17 grams; water, 1 liter.

[7] This medium consisted of: Peptone, 5 grams; KH_2PO_4, 1 gram; $MgSO_4$, 1 gram; dextrose, 20 grams; agar, 17 grams; and water, 1 liter.

7. Five single ascospore isolates were grown on Brown's "synthetic potato-dextrose" agar[8] and on the same medium plus M/7000 potassium permanganate. Duplicate plates of each culture on the two media were placed at 10°, 15°, 20°, 25°, and 30° C. Here again only line A36-1-V sec formed perithecia, and these only in small numbers at 15°, 20°, and 25° C.

8. Cultures were grown on potato-dextrose agar to which wheat germ was added. The medium was sterilized in the Arnold sterilizer by steaming for one hour on each of three successive days. An ether extract of the wheat germ was also added to cultures growing on potato-dextrose agar. Both the oil and the wheat germ stimulated a very heavy growth of aerial mycelium but no perithecia were formed.

9. Several workers, notably Stevens (51, 52, 53), have succeeded in stimulating the production of perithecia in various ascomycetes by irradiating cultures with ultra-violet light.

In a preliminary experiment eight ascospore isolates from a single ascus were grown on Coons' agar and Leonian's agar in petri dishes. When the cultures were 19 days old, they were irradiated for 45 seconds with a small mercury-vapor arc lamp at a distance of 15 centimeters. This treatment did not result in perithecial formation.

A more extensive experiment was then made. Twelve lines were used, six of which formed perithecia on Coons' agar and potato-dextrose agar under ordinary laboratory conditions. The cultural types of the other six cultures are indicated in Table 6. These lines were grown on five sets of duplicate plates containing 30 cc. of potato-dextrose agar. Two days after inoculation one set was placed in a box to exclude the light, one was left in the laboratory in diffused daylight, and the other three were irradiated for periods of 30 seconds, one minute, and two minutes, respectively. The covers were removed during the irradiation period and the plates placed at a distance of four and one-half feet from the arc. The source of the ultra-violet light was an Eveready carbon arc, model 49m, drawing 60 amperes at 115 volts. A therapeutic "C" carbon was used, developing a high intensity of ultra-violet light of wave lengths ranging from 3,000 to 4,000 Angström units. After irradiation, the plates were placed in the laboratory with the second lot.

The immediate effect of irradiation was a checking of the growth of the colonies for about two days. After this time, they started to grow again and apparently developed a little more rapidly than did the non-irradiated cultures, the irradiated colonies covering the surface of the agar about a day later than the same culture which had not been

[8] This medium consisted of: Glucose, 2 grams; asparagin, 2 grams; K_3PO_4, 1.25 grams; $MgSO_4$, 0.75 gram; agar, 17 grams; and water, 1 liter.

irradiated. The only other noticeable effect of irradiation was a slight reduction in the color intensity of the cultures and a reduction in the number of perithecia formed by those lines which characteristically produced the perfect stage in culture.

Table 6

The Effect of Ultra-Violet Irradiation on Perithecial Development in Twelve Lines of *Gibberella saubinetii*

Line	Cultural type	Treatment and perithecial development				
		Darkness	Diffuse daylight	Ultra-violet irradiation		
				½ minute	1 minute	2 minutes
A43-4-VIsec	K	Ab*	Ab	F* near center	I* near center	I near center
A36-1-Vsec	K	Ab	Ab	F on surface I in agar	F on surface I in agar	F on surface I in agar
A43-4-I-I	K	F	F	tr.	tr.	tr.
OA39-III	K	Ab	Ab	0	0	0
A36-1-VIIIsec	K	F	F	0	0	0
9	K	Ab	Ab	F on surface Ab in agar	F on surface Ab in agar	F on surface Ab in agar
A35-1-V	G	0	0	0	0	0
A35-1-II	D	0	0	0	0	0
A38-1-III	C	0	0	0	0	0
A43-4-VI	A	0	0	0	0	0
A43-4-I	A	0	0	0	0	0
A38-1-I	I	0	0	0	0	0

* 0 = no perithecia; F = small numbers of perithecia; I = moderate numbers; Ab = abundant.

The appearance of perithecia in these cultures was delayed about a day by irradiation, but there appeared to be no marked differences due to different lengths of the irradiation period. The relative numbers of perithecia formed in each culture are shown in Table 6. It will be noted that lines 9 and A36-1-Vsec, while forming relatively few perithecia on the surface of the colony, did produce them abundantly in the agar. This was characteristic of these two lines when not irradiated, and the failure of the ultra-violet rays to check the development of the fruiting bodies might be interpreted as due to a failure to penetrate to the submerged mycelium, which developed unharmed. It will be seen that none of the sterile lines responded to this treatment by forming the perfect stage.

More or less artificial methods of stimulation having failed to induce the formation of perithecia, it was decided to try a combination of conditions which more closely approximated those found in nature. The following experiment was therefore made.

Sections of mature corn stalk were cut about four inches long and placed in wide-mouth glass jars with screw caps. Two such pieces were placed in each jar, one piece having the epidermis removed, while

the other was left intact. Fifty cubic centimeters of water was added to each of half of the jars and 100 cubic centimeters to each of the others. The jars were sterilized for an hour in the autoclave at 15 pounds pressure. After standing for a week to allow the appearance of possible contaminants, twenty-four jars, half containing 100 cc. of water and half 50 cc., were then inoculated with cultures of *Gibberella saubinetii*. Four lines were used: A38-1-I, type I; A35-1-VI, type C; A43-4-IV, type A (normal), and A36-1-Vsec, type K. After seven days, spores from a culture of *Penicillium* were added to half of the jars. These jars were divided into three lots and subjected to different temperatures as shown in Table 7.

Table 7

The Effect of Moisture, Temperature and Contaminants on the Formation of Perithecia* by four Lines of *Gibberella saubinetii*

Line	High moisture						Low moisture					
	Contaminated with penicillium			Not contaminated			Contaminated with penicillium			Not contaminated		
	2°C.	17°C.	r.t.†	2°C.	17°C.	r.t.†	2°C.	17°C.	r.t.†	2°C.	17°C.	r.t.†
A38-1-I	0	0	0	0	0	0	0	0	0	0	0	0
A36-1-Vsec	I	Ab	Ab	I	Ab	I	I	Ab	Ab	Ab	Ab	Ab
A35-1-VI	0	0	0	0	0	0	0	0	0	0	0	0
A43-4-IV	0	F	F	F	F	F	F	F	F	0	F	0

* 0 = no perithecia; F = small numbers of perithecia; I = moderate numbers; Ab = abundant perithecia.

† r.t. = room temperature (20° C. to 25° C.).

The results of this experiment, presented in Table 7, show that these conditions failed to induce perithecial formation in the variant types A38-1-I and A35-1-VI. Line A36-1-Vsec, which produces perithecia on artificial media, formed numerous fruiting bodies under all conditions in this experiment except that the numbers were slightly reduced in some of the cultures kept at 2° C. The normal type, A43-4-IV, produced a few perithecia containing mature ascospores under all except three of the conditions to which it was subjected. This experiment indicates that the variant types may lose their ability to form perithecia; but the results are not conclusive, because the variant and normal lines used were from different collections.

Early attempts to induce the formation of perithecia by growing cultures on sterilized grains and placing the inoculated grain in sand yielded negative results. However, during the pathogenicity tests on wheat, it was noted that several cultures formed perithecia on the oat hulls which had been added to the sand as the medium upon which the inoculum was grown. It was further observed that three parent lines

formed perithecia, while a variant from each did not. The following experiment was then made to test the perithecia-forming ability of cultures under such conditions, with the particular object of comparing the behavior of variant lines with those which still appeared normal.

Sixty-two cultures were grown for a week on sterilized oat hulls in Erlenmeyer flasks, and a vigorous mycelial growth developed. A liberal amount of the oat-hull culture was then stirred into sterilized sand in duplicate two-inch pots. A piece of cheese cloth was placed over each pot to reduce the possibility of cross contamination. The pots were sunk about three quarters of an inch in the sand on a greenhouse bench and kept thoroly wet by sprinkling with water.

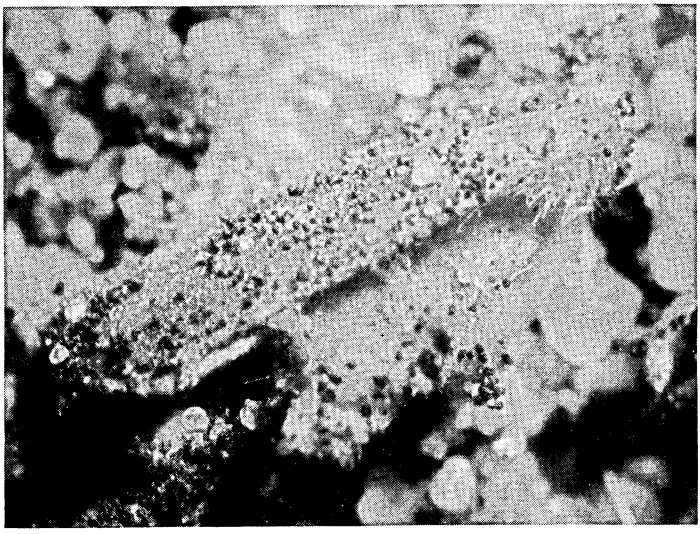

Fig. 1. Perithecia of *Gibberella saubinetii* on oat hulls. The culture (A43-4-IV) was grown on sterilized oat hulls for a week and then this culture was mixed with sterilized sand in pots and placed in the greenhouse. Perithecia appeared in 12 days. X10.

Observations were made after 12 and 22 days. Perithecia were found in the same cultures on both dates. When the last observations were made, the surface of the sand was thoroly examined with a hand lens and with the unaided eye. If no perithecia were found on the surface the sand was stirred up and the buried oat hulls examined. In no case were perithecia found buried in the sand if none were found on the surface. Figure 1 is a picture of perithecia formed on oat hulls in the greenhouse.

The results, presented in Table 8, show that only one line, A36-1-VII, designated as a variant which did not produce perithecia in culture.

produced the perfect stage under the conditions of this experiment. On the other hand, only six of 26 lines which appeared to be normal failed to form perithecia. Lines A36-1-VIII-h-7a and A36-1-VIII-h-1a, which originated from single cells of a conidium, failed to produce perithecia in this experiment, but did so on agar, as described in a later section of this paper.

Table 8

Production of Perithecia by Lines of *Gibberella saubinetii* on Oat Hulls in Sand in the Greenhouse

No perithecia produced		Few perithecia		Moderate numbers of perithecia		Abundant perithecia	
Line	Type*	Line	Type	Line	Type	Line	Type
A12-1-II	B	A39-1-I	A	9	K	A36-1-Vsec	K
A12-1-IV	F	A36-1-VII	C	A35-1-IV	A	A36-1-Vsec-H-2a	K
A12-1-V	D	A42-1-IV	A	A35-1-V	A	A39-1-IV	A
A12-1-VII	E	A42-4-I	A	A36-1-Vsec-H-5b	K	A39-1-V	A
A12-1-VIII	E	A43-4-1-I	K	A38-1-VIII	A		
A35-1-I	D	A43-4-I-I-h-5a	K	OA39-III	K	A42-1-II	A
A35-1-II	D	A43-4-I-I-h-1a	K	A43-4-III	A	A42-4-VII	A
A35-1-III	C	A43-4-V	A	A43-4-Vsec	A	A43-4-I	A
A35-1-VI	C	A43-4-VIII	A	A43-4-VIsec	K	A43-4-II	A
A36-1-III	A			A43-7-II	A	A43-4-IV	A
A36-1-IV	A					A43-5-VI	A
A36-1-V	A					A43-7-IV	A
A36-1-VI	C						
A36-1-VIII	C						
A36-1-VIIIsec	K						
A36-1-VIII-h-7a	K						
A36-1-VIII-h-1a	K						
A38-1-I	I						
A38-1-II	H						
A38-1-III	C						
A38-1-IV	D						
A38-1-V	D						
A38-1-VI	C						
A38-1-VIIIsec	G						
A39-1-II	E						
A39-1-VI	A						
A39-1-VII	A						
A39-1-VIII	D						
A43-4-IVsec	H						
A43-4-IIIsec	J						
A43-4-IIsec	G						
A43-4-VI	A						

* These types are described in the section on variation.

That variants arising in cultures may lose their ability to form perithecia is shown more definitely by the behavior of lines A43-4-IIsec, A43-4-IIIsec, and A43-4-IVsec. None of these lines produced perithecia under the conditions of this experiment, while the parent lines A43-4-II, A43-4-III, and A43-4-IV formed the perfect stage in abundance. These results confirm the observations made in the pathogenicity experiment on wheat, where the same parent lines were observed to produce perithecia while the variants did not.

THE FORMATION OF PERITHECIAL VARIANTS

It was frequently observed that some cultures of *Gibberella saubinetii,* when grown on Coons' or potato-dextrose agar in Erlenmeyer flasks, formed small groups of perithecia-like structures. These were usually sunken below the surface of the agar. Many of these structures were examined, but they always were empty.

Fig. 2. Line A43-4-VIsec originating as a sector in line A43-4-VI. The sector produces perithecia containing mature ascospores.

Finally such a group was removed with the inoculating needle and transferred to fresh agar in a flask. As the colony grew, a sector developed which bore numerous perithecia. (See Fig. 2.) By repeating this process, three such variants were secured which produced perithecia and mature ascospores in culture.

Many times no sector developed. In one such case the perithecia seemed to increase in numbers on the old piece of inoculum. These were examined and a few mature ascospores were found in one perithecium. Some of these were isolated and two of them grew. The resulting lines were designated A43-4-I-I and A43-4-I-II. The former is described in Table 9. It is sufficient here to state that they produced, when old, hyphae consisting of short, heavy-walled, blue cells and a few bodies resembling small perithecia. These were never found to contain ascospores.

Due to the manner in which these perithecial variants originated, it is the opinion of the writer that small "patch" variants having the capacity of producing perithecia in culture may arise which, when isolated and transferred to fresh agar, may grow rapidly enough to appear as a sector in the resulting colony.

Once established, these variants appear to be quite stable. They were propagated many times, both from conidia and ascospores, and, with two exceptions, always appeared true to type. In one of these exceptional cases, a colony of the variant type C bearing no perithecia was produced from a single ascospore isolated from line A36-1-VIIIsec. It will be remembered that this type was found once among the isolates of naturally occurring ascospores. Seven other ascospores from the same ascus yielded cultures true to the parental type. In the other case a single ascospore isolate from line A43-4-VIsec produced a small sector resembling the original parent isolate which did not produce perithecia in culture.

The cultural characteristics of six perithecial lines are given in Table 9. This group consisted of lines A36-1-Vsec, A36-1-VIIIsec, and A43-4-VIsec, which arose in the manner described above; A43-4-I-I, which was isolated from a perithecium occurring on old inoculum after transfer to new medium; OA39-III, which produced perithecia when first isolated from naturally occurring perithecia; and culture 9, secured from Prof. L. R. Jones. The relative growth rates of these cultures at three temperatures is given in Table 10. It is sufficient here to state that each of these cultures was easily distinguishable from the rest by even a casual observer.

Table 9

Cultural Characteristics of Six Perithecial Lines of *Gibberella saubinetii* on Potato-Dextrose Agar. Cultures 25 Days Old

Line	Bottom	Top		Topography	Perithecia
	Color	Mycelium			
		Color	Growth type		
9	Ox-blood red	Pinkish cinnamon	Cottony; uniform over entire colony	Three short radial furrows in center	Numerous near center; scattered over rest of colony; developing abundantly if aerial mycelium is removed. Also abundant in agar, about 2 mm. deep.
A43-4-VI sec	Eugenia red	Eugenia red	Aerial mycelium very scant, except in center	About 10 radial furrows extending nearly to margin	Numerous all over surface of colony; none in agar.
A36-1-V sec	Ox-blood red	Eugenia red	Cottony; uniform over entire colony	Level; no radial furrows	Numerous all over colony; hidden by aerial mycelium. Few in agar 2 mm. deep.
A36-1-VIII sec	Carmine	White; later appearing ox-blood red	Felt-like; shrinking and adhering to colony in older cultures	Four long radial furrows; often splitting agar	Scant; scattered over surface of agar. None in agar.
OA39-III	Eugenia red	Ox-blood red, few white aerial hyphae	Appressed except for few aerial hyphae	12 to 15 radial furrows and 3 concentric furrows	Numerous on surface; rudimentary—no asci.
A43-4-I-I	Orange-pink; becoming deep purple when old	Purple; few orange-pink aerial hyphae; mycelium composed of short, thick-walled cells	Appressed except for few aerial hyphae	About 12 shallow radial furrows; one deep concentric furrow near center	Numerous on surface; rudimentary—no asci.

Table 10

Diameter, in Centimeters, of Four-Day-Old Cultures of *Gibberella saubinetii* at Three Different Temperatures

Temperature	A43-4-VI sec		A43-4-I-I		A36-1-V sec		A36-1-VIII sec		OA39-III		9	
	P.D.A.	Coons'	P.D.A.	Coons'	P.D.A.	Coons'	P.D.A.	Coons'	P.D.A.	Coons'	P.D.A.	Coons'
17° C.	4.25	2.55	3.20	1.75	5.20	2.25	4.35	2.55	3.50	2.20	4.60	2.05
24° C.	6.20	3.70	4.20	2.50	7.70	3.95	6.15	4.20	4.20	2.65	6.95	2.30
28° C.	5.25	4.20	4.30	2.30	7.35	5.00	6.40	4.10	4.30	2.60	6.45	3.05

HOMOTHALLISM IN *GIBBERELLA SAUBINETII*

Early in these investigations single ascospore isolations were made from line 9. These isolates were in every respect apparently identical with the parent, indicating that the fungus is homothallic. Subsequently, an article by Bennett (5) appeared in which he reported the same to be true of isolates of the organism in England. Numerous isolations of ascospores and of conidia showed this to be true, with two exceptions, of all four cultures which produced ascospores in culture. The two exceptions, as noted previously, may be regarded as variants.

The literature contains several reports of apparent delayed segregation of factors for sex in the fungi. Dodge (16) has demonstrated that segregation of factors for sex and conidial production may take place in the first or second division of the nucleus in the ascus of *Neurospora* spp. Lately Buisman (9) has shown that single conidial isolates taken from a single ascospore culture of *Ceratostomella fagi* Loos would not produce perithecia until the cultures were mated, altho the original single ascospore culture did produce perithecia. She found that of eight single-conidial cultures four were complementary to each of four others; that is, perithecia were produced in 50 per cent of the combinations. As only eight conidia were isolated, it seems remarkable that this theoretically perfect assortment was found. However, this work might be interpreted as a case of delayed segregation of sex factors.

In *Ustilago zeae* (Beckm.) Ung. Christensen (13) has found "solopathogenic" lines in which segregation of sex factors did not take place in the promycelium, as it does in most cases in that fungus. Such lines were carried for some time in culture and through several inoculations into, and subsequent reisolations from, the host plant. Finally, segregation seemed to take place in some cultures, resulting in typical haploid lines which had to be mated with another line of opposite sex before normal parasitism was possible. This, too, appears to be a case of delayed segregation.

In view of the apparent probability that this phenomenon takes place in some fungi, it was thought possible that it might also occur in *Gibberella saubinetii.* At least it was thought advisable to grow cultures that arose from single cells before concluding that the lines studied were homothallic.

Accordingly, hyphal tips were cut from the germ tubes of a number of germinating conidia and ascospores, using the technic described under materials and methods. The exact number of hyphae so isolated was as follows: line 9, 15 hyphae from nine ascospores, 15 hyphae from seven conidia; line A36-1-Vsec, 23 hyphae from nine ascospores, 19 hyphae from nine conidia; line A36-1-VIIIsec, 24 hyphae from eight ascospores, 10 hyphae from seven conidia; line A43-4-VIsec, 10 hyphae from five ascospores, 12 hyphae from nine conidia; line OA39-III, 17 hyphae from seven conidia; line A43-4-I-I, 19 hyphae from 10 conidia.

The resulting cultures were grown on slants of both potato-dextrose and Coons' agar. All produced perithecia and mature ascospores on Coons' agar except lines OA39-III and A43-4-I-I. On potato-dextrose agar the results were the same, except that hyphal tip cultures from ascospores of line A38-1-Vsec produced no perithecia, and four cultures from conidia of line 9 failed to do so. This behavior has not been investigated, but the appearance of the perfect stage when these lines were grown on Coons' agar establishes the fact that the factors for sex were present in all of the cultures arising from single cells of either conidia or ascospores of lines 9, A36-1-Vsec, A36-1-VIIIsec, and A43-4-VIsec. Lines A43-4-I-I and OA39-III, it will be remembered, were never observed to produce ascospores. The cultures arising from single hyphal tips from conidia appeared to be identical with the parent cultures.

These results lead to the conclusion that the lines investigated, except A43-4-I-I and OA39-III, were homothallic. The possibility remains that if the last two cultures were mated with each other or with other cultures the rudimentary perithecia would produce ascospores. At the time this paper was written this had not been done.

CYTOLOGICAL STUDIES

While no detailed investigations of the cytology of *Gibberella saubinetii* were carried out, a few observations were made of the nuclear conditions of the conidia and ascospores, as well as of the development of the ascus and ascospores.

Conidial material for these studies was placed in a drop of water on a slide smeared with fresh egg albumin. When the water was nearly evaporated, the spores were killed by exposing to the vapor of Flem-

ming's weaker solution. The slides were placed four hours in 4 per cent iron alum as a mordant. They were then stained for 24 hours in one-half per cent haematoxylin and destained in two per cent iron alum.

This schedule was not found suitable for ascospores because the haematoxylin could not be removed from the mature spores. After some experimenting the material was finally stained satisfactorily by the following method. The perithecia were gently crushed in a drop of water on a slide smeared with fresh egg albumin. After drying, the material was killed by placing for 10 minutes in a mixture of two parts of absolute alcohol to one of acetic acid. Four per cent iron alum was used as a mordant and one-half per cent haematoxylin as a stain. The slides were destained in a saturated solution of picric acid. The destaining process took from two to three hours.

An examination of the material revealed that the formation of the ascospores takes place in the manner usually described as typical for the ascomycetes. (See Plate V, Figs. A to G.) Young asci contain a single nucleus (Fig. A); later stages have two (Fig. B), four (Fig. C), and eight (Fig. D) nuclei. The young ascospores are formed around these eight nuclei (Fig E). Subsequently this nucleus divides to form the four nuclei of the mature spore (Figs. F and G). All of the spores examined within the ascus contained only one nucleus in each cell, but a few of those which had escaped were found to have two nuclei in some of the cells (Figs. K and L). It is possible that a division of the nucleus preceded germination, but Figure M shows a germinating spore with only one nucleus in the germinating cell.

Some of the conidia were also found to have more than one nucleus in each cell. This observation may be contrasted with that of Dickinson (17), who found only one nucleus per cell in conidia of *Fusarium fructigenum* Fries. and *F. vasinfectum* Atk. var. *lutulatum*. It seems from the appearance of some of these conidia (see Figs. U, V, W, and X) that septa were about to be formed between some of the nuclei present in one cell. On the other hand, the conidium shown in Figure T appears to be mature, but two of the cells each contain two nuclei.

It is apparent from these studies that some of the germ tubes cut from the individual cells of ascospores or conidia may have come from cells containing more than one nucleus. However, the number of cells containing more than one nucleus is small, especially among the ascospores, and one is forced to assume that most of the hyphae which were isolated, as described in the previous section, arose from uninucleate cells. It is highly probable, however, that some did arise from cells containing more than one nucleus. As no difference was noted in the

resulting cultures, we have no clue as to the possible significance of the irregular nuclear behavior observed.

PATHOGENICITY STUDIES

A measure of the pathogenicity of various cultures of *Gibberella saubinetii* was made by testing their ability to cause head blight of wheat and barley in the field and seedling blight of wheat in the greenhouse. Seedling blight tests on corn were made under controlled conditions of light and temperature.

Field Studies

Varieties of wheat and barley were grown and inoculated in the field during the summers of 1931 and 1932. Due to the dry weather during those seasons, no infection occurred and no notes were taken.

In 1933 the tests were made under a muslin cage which insured a relative humidity high enough to permit infection to take place. Under these conditions considerable lodging occurred, and as the experiment was made only once, the results are not considered reliable enough to be presented here in detail. In this study five varieties of barley and four of wheat were inoculated with nine variant lines of *Gibberella saubinetii*. The results, while inconclusive, indicated that there were differences in pathogenicity among the strains of the pathogen as well as differences in susceptibility among different host varieties.

Seedling Blight Experiments on Corn—Methods

In the seedling blight tests on corn, three selfed lines were used.[9] These were Rustler, culture 20, selfed eleven years; Golden Bantam, culture 72, selfed six years; and Minnesota 13, culture 11, selfed nine years. It may be assumed that these lines were relatively homozygous.

The seeds were surface disinfected by dipping them in 70 per cent alcohol and then soaking four minutes in concentrated sodium hypochlorite solution. The sodium hypochlorite was removed by washing in cold tap water. It is to be expected that some bacterial contamination resulted from this procedure, but the fungi were quite effectively removed, less than one per cent of the seedlings being visibly contaminated with *Penicillium* or other fungi.

The cultures of *Gibberella saubinetii* used in these experiments were grown on sterilized wheat and oats mixture in Erlenmeyer flasks. When these cultures were five days old, a heavy growth of mycelium was present. Aqueous suspensions of inoculum were then made by washing

[9] The seed corn was furnished by Dr. Iver Johnson of the Division of Agronomy, Minnesota Agricultural Experiment Station.

the cultures with sterile distilled water and straining through one thickness of sterile cheese cloth. The seeds were inoculated by allowing them to stand four or five minutes in this suspension of inoculum.

The spore and mycelial suspensions used were not standardized with respect to the total amount of inoculum present. This was not done because some of the cultures produced practically no conidia and an adequate basis was lacking for rendering these comparable with those which did produce conidia. Each of the cultures used produced abundant mycelium on the wheat and oats medium and the suspensions made contained innumerable fragments of hyphae as well as conidia in some cases.

Altho experiments were not made to prove the point, it is felt that if the inoculum were diluted a great deal differences in dilution might make a difference in the amount of disease produced. However, it seems that beyond a certain point increases in the concentration of inoculum would not be likely to make such a difference. It is felt that the suspensions used afforded a maximum of inoculum in each case.

That the mycelial fragments constituted effective inoculum is shown by the experimental results given in Figure 5A. These lines produced practically no conidia but were highly pathogenic. On the other hand, in the results shown in Figure 7A, line OA39-III was the only one which produced distinctly fewer conidia than the others. Yet it is more pathogenic than line A43-4-I-I on two selfed lines of corn.

That the difference caused by concentration of inoculum probably did not play an important part in the present experiments is indicated by comparing experiments A and B shown in Figure 7. In the experiment on wheat (B) the fungus was grown on oat hulls which were added to the sand, thus insuring a maximum of inoculum. Yet the relative pathogenicity of the cultures is approximately the same on wheat as on corn. It is realized, of course, that wheat and corn are not strictly comparable.

Thirty-two seeds of each line of corn, inoculated with the same culture, were planted in triplicate flats filled with autoclaved sand to a depth of about four inches. The seeds were uniformly spaced, one inch by two inches, and planted uniformly one inch deep.

Immediately after planting, the flats were placed in a refrigerated room maintained at about 17° C. It has been shown by McIndoe (37) that soil temperatures of about 15° C. are most suitable for corn root rot tests with *G. saubinetii.* In the present studies a temperature of 17° C. was found satisfactory for differentiation of strains on the basis of their ability to cause seedling blight.

Illumination was furnished by two 500-watt Mazda lamps, which were kept turned on about twelve hours a day. Equal amounts of water were applied to the flats each day, and the flats were moved every day to overcome as much as possible the effects of different locations in the room.

Notes were taken at the end of three weeks. The method of recording seedling blight damage was devised to furnish as accurate as possible a means of measuring the damage done. At the time the notes were taken on the first experiment there were selected a series of ten seedlings which were photographed and used as a standard for taking notes on later experiments. These seedlings represented a complete range of possible degrees of injury.

In taking the notes, each seedling or seed was dug up and given a numerical score corresponding to that of a similar plant in the standard types. These types are shown in Figure 3. Thus, if a seedling was perfectly healthy, it was scored 1; if it had been killed before the primary shoot emerged from the seed coat, it was scored 10, and so on. It was very easy to recognize the ten types, and it is believed that the personal element was thus reduced to a minimum.

The score for the check flats was raised above the theoretical perfect score of 32 for 32 plants, due to the fact that in each case several seeds did not germinate. These seeds were, of course, scored 10. Assuming that each lot of 32 seeds taken at random from the same population would contain on the average the same number of non-viable seeds, it is evident that some of the injury ascribed to each culture of the fungus was actually due to the lack of germination of the seed. Thus, the actual injury caused by the fungus is less than that which was recorded. The difference, however, would not be equal to the amount by which the average score for a given set of check flats exceeds 32, since if the non-viable seeds had germinated they would be subjected to the same injury as the others. Thus, the more pathogenic the culture, the smaller the error due to non-viability becomes; and the actual differences due to the cultures would become greater than they now appear.

The scores for the 32 seedlings in each replicate were totaled and the figures analyzed statistically by the analysis of variance method proposed by Fisher (22). The figures given in the tables represent the means of three replications. These figures will be referred to as an index of pathogenicity. To put the results on the basis of a single plant for purposes of comparison with Figure 3, the mean totals are divided by 32 and presented in the same table. The numbers thus obtained range from 1 to 10 and are referred to as the index of pathogenicity on a single plant basis or units of pathogenicity.

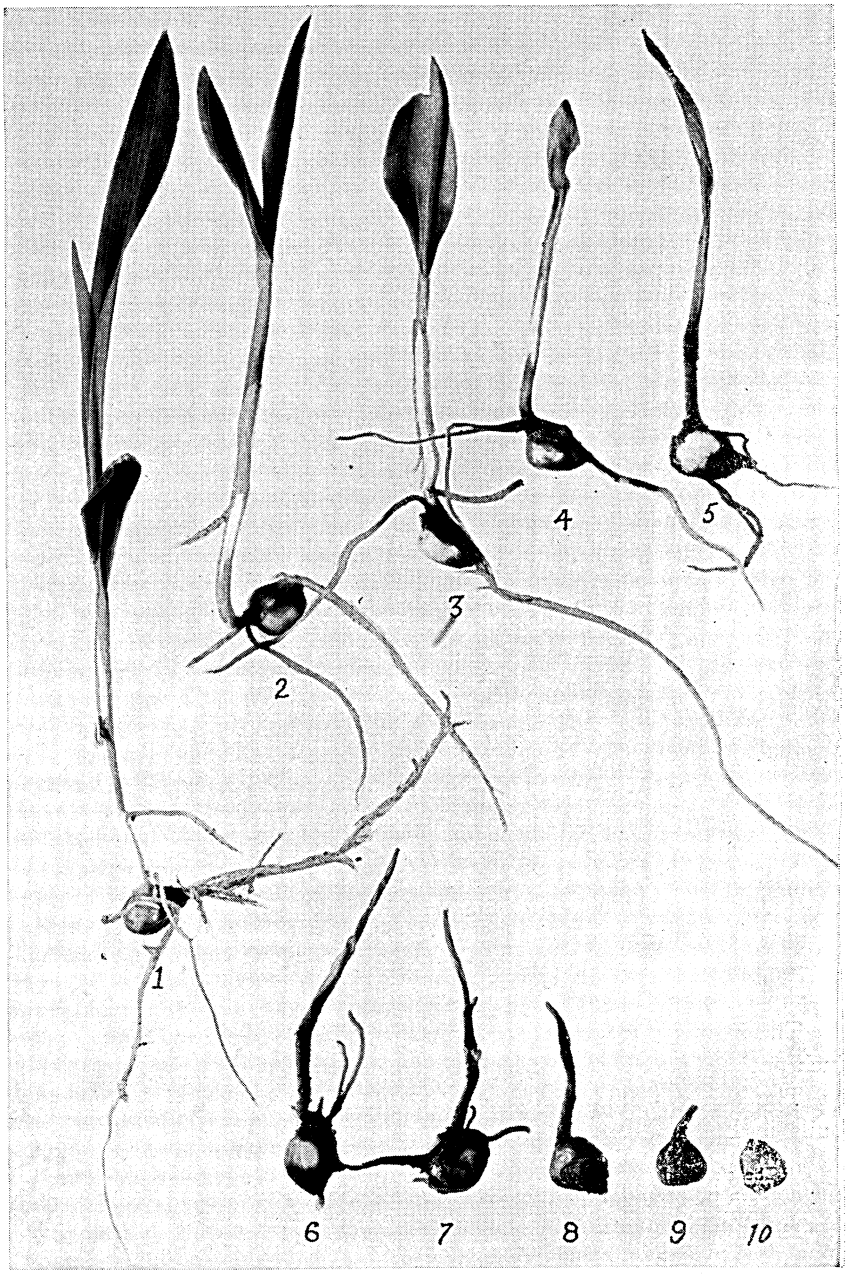

Fig. 3. Standard types used in scoring blighted corn seedlings.

To further facilitate the examination of the data, they are represented graphically in Figures 5, 6, and 7. The lines used in a single experiment are not listed in a single order for all three host varieties but in the order of their relative pathogenicity. This makes it possible to group cultures which are not significantly different[10] in pathogenicity. This has been done, and the lines which are not significantly different are enclosed in brackets to the left of each figure. It is also possible, by observing the relative order of the cultures on each line or variety, to locate quickly differences in the reaction of two varieties to a given culture. To check the significance of such interactions reference must be made to the tabulated data.

The number of lines which could be tested by this method was limited by the space available in the constant temperature room, and since it was desired to test a number of series of lines most of the experiments were made only once. However, to test the reliability of the method the experiment on the perithecial lines was made twice. The results of the analysis of variance shown in Table 17 show that there was no significant difference between the two experiments, since the variance for experiments was less than the variance for error. Likewise no significant difference between the interaction of lines x experiments was found as the value of z did not reach the five per cent point. However, there was a significant difference in the reaction of varieties. An examination of Table 16 reveals that Rustler was more severely injured in the second experiment than in the first by lines OA39-III and A43-4-I-I. On the other hand, Golden Bantam was less severely attacked in the second experiment than in the first by lines A36-1-VIII sec and OA39-III. Thus a total of only four comparisons in eighteen were significantly different in the two experiments, indicating that the method is a fairly reliable test of the pathogenicity of the cultures.

Seedling Blight Studies on Wheat—Methods

The seedling blight experiments on wheat were carried out in four-inch pots of autoclaved sand in the greenhouse.

The cultures for these experiments were grown on sterilized oat hulls in Erlenmeyer flasks. When the cultures were seven days old,

[10] The standard of significance here used is based upon the standard error of the difference between two means. This is represented by the formula $\sqrt{\frac{\sigma^2 \times 2}{N}}$ where the standard deviation squared (σ^2) is equal to the mean square for error found in the analysis of variance in each experiment and N equals the total number of replications in a given mean. The one per cent point was selected as the minimum level of significance. The minimum significant difference between any two means is obtained by multiplying the standard error of the difference between two means by the "t" value corresponding to the number of degrees of freedom for error at the one per cent point. (See Fishers' Table of "t" (22).)

approximately equal amounts of the mixture were stirred into the top inch of sand in each pot. The seeds were then scattered on the surface of the sand and fungus mixture and covered with about three-quarters of an inch of autoclaved sand.

Three varieties of wheat, Prelude, Pentad, and Marquillo, were used. The seeds were disinfected in the same manner as was the corn seed. Twenty-five seeds of a single variety were planted in each of four pots containing the same culture. The pots were placed in a random manner on the greenhouse bench, and notes were taken eighteen days after planting.

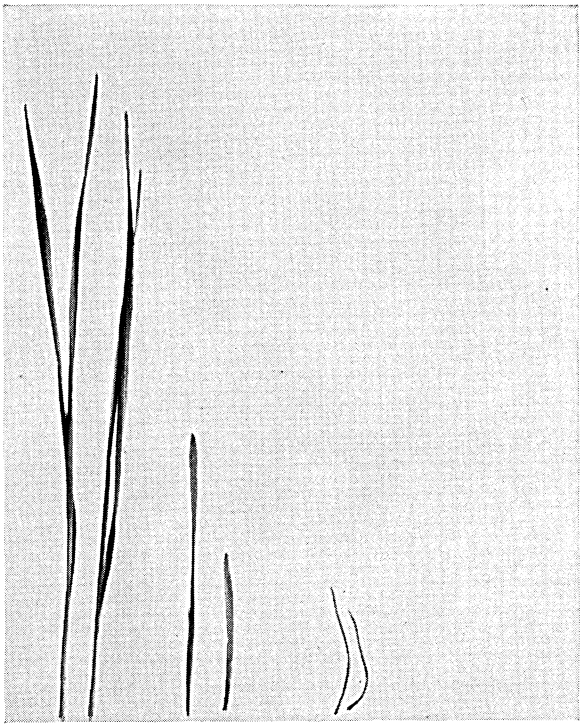

Fig. 4. Standard types used in scoring blighted wheat seedlings.

On the basis of the appearance of the parts above the soil, four degrees of pathogenicity were recognized. The types are shown in Figure 4. Type 1 is represented by apparently healthy plants; type 2 by stunted individuals; type 3 by those which emerged, but which were killed later; and type 4, obtained by difference, by those plants which never emerged from the soil. These plants were scored from

one to four according to the types described above, and the score of the twenty-five plants in each replicate totaled and analyzed by Fisher's analysis of variance method. The fewer number of classes recognized no doubt makes this experiment less accurate than the tests on corn, but it was felt that to try to recognize ten degrees of injury on small plants would involve considerable error and that four would serve nearly as well.

Results of Seedling Blight Tests

1. Differences between sister ascospore isolates.

The lines used in this test originated from ascospores taken from a single ascus found in a perithecium on corn stubble. These were culturally identical on potato-dextrose agar and Coons' agar as previously described, and the following experiment was made to find if they were also identical pathogenically. The test was made as soon as possible after isolation, the cultures being grown for only about a week on potato-dextrose agar slants before the wheat and oats medium was inoculated. Since the fungus remains fairly stable on potato-dextrose agar, it is probable that no variation took place before the test was made.

The results are presented in Table 11 and Figure 5A. The values of z for lines and for varieties were found to exceed the one per cent point. Similarly, it was found that the value of z for the interaction of varieties by lines exceeded the one per cent point. However, since the latter differences are not of great importance in these experiments, the purpose of which is to demonstrate differences between cultures on each variety, they will not be referred to in the following discussions.

Table 11

Index of Pathogenicity on Three Selfed Lines of Corn of Eight Single Ascospore Lines from One Ascus of *Gibberella saubinetii*

Line*	Mean index for 32 plants†‡			Mean index for 1 plant§		
	Rustler	Gold. Bant.	Minn. 13	Rustler	Gold. Bant.	Minn. 13
A43-4-I	271.00	276.00	237.67	8.47	8.62	7.43
A43-4-II	272.00	283.33	262.67	8.50	8.85	8.21
A43-4-III	274.33	280.00	243.67	8.57	8.75	7.61
A43-4-IV	283.00	284.33	275.67	8.84	8.89	8.61
A43-4-V	277.00	291.33	260.00	8.66	9.10	8.12
A43-4-VI	276.00	280.00	257.67	8.62	8.75	8.05
A43-4-VII	299.00	260.67	241.00	9.34	8.15	7.53
A43-4-VIII	272.00	276.00	265.67	8.50	8.62	8.30
Check	51.00	69.00	96.00	1.59	2.16	3.00

* Value of z for lines exceeds the one per cent point.
† Value of z for varieties exceeds the one per cent point.
‡ Minimum significant difference between two means for 32 plants, 24.50 units.
§ Minimum significant difference between two means for one plant, 0.766 unit.

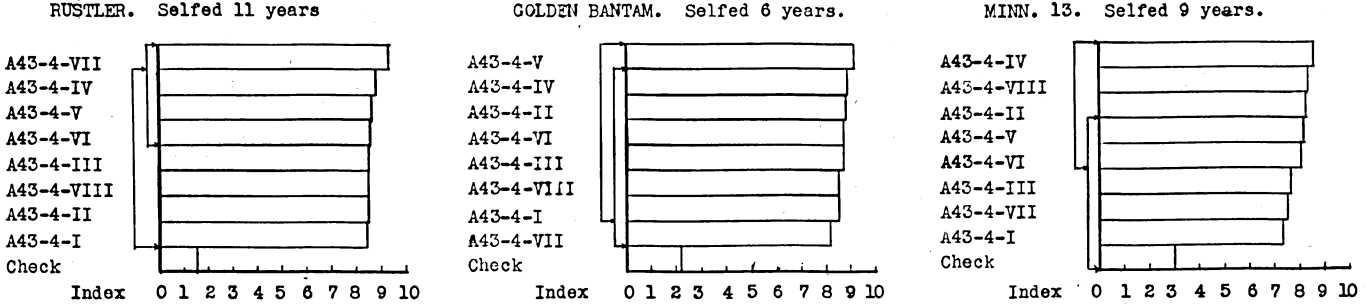

Fig. 5A. Relative pathogenicity on three selfed lines of corn of eight single ascospore isolates of *Gibberella saubinetii* from a single ascus. Index on single plant basis. Minimum significant difference between lines—0.766 unit.

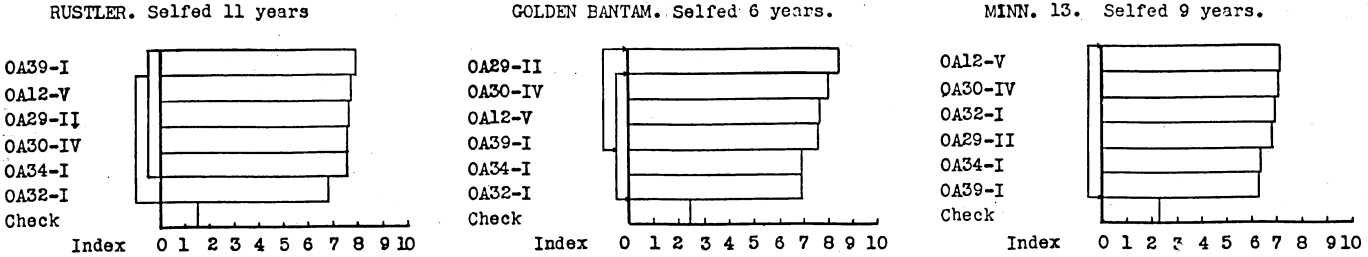

Fig. 5B. Relative pathogenicity on three selfed lines of corn of six single ascospore isolates of *Gibberella saubinetii* from different collections. Index on single plant basis. Minimum significant difference between lines—1.087 units.

It can be seen by examining Figure 5A that some of the lines differed in pathogenicity by small, tho statistically significant, amounts. On Rustler, seven lines are not significantly different from one another, while one is more pathogenic than four of the others but not significantly more so than the other three. On Golden Bantam only the most pathogenic and the least pathogenic are significantly different from each other, while on Minnesota 13 the first three and the last three constitute two groups which are significantly different pathogenically.

On the basis of the three lines of corn we are thus able to distinguish the following four pathogenic groups: (a) A43-4-VII; (b) A43-4-II, A43-4-IV, and A43-4-VIII; (c) A43-4-I and A43-4-III; and (d) A43-4-V. Line A43-4-VI is not significantly different from the other lines on any of the three lines of corn used. If more lines of corn had been used, it is possible that more forms could have been distinguished in this group.

2. Differences between ascospore isolates from different collections. These lines were taken from among those described in the second experiment on the cultural types of field isolates. They were selected to represent the mean and extremes of color intensity represented by the isolates in that experiment. Thus lines OA39-I and OA34-I were deep red, OA32-I and OA12-V intermediate, and OA30-IV and OA29-II pale pink.

Table 12

Index of Pathogenicity on Three Selfed Lines of Corn of Six Single Ascospore Lines of *Gibberella saubinetii*

Line*	Mean index for 32 plants†			Mean index for 1 plant‡		
	Rustler	Gold. Bant.	Minn. 13	Rustler	Gold. Bant.	Minn. 13
OA30-IV	242.33	255.67	225.00	7.57	7.99	7.03
OA32-I	218.00	221.00	222.33	6.81	6.91	6.95
OA12-V	247.67	244.67	226.33	7.74	7.65	7.07
OA39-I	255.33	243.33	200.33	7.98	7.60	6.26
OA34-I	241.00	221.00	201.33	7.53	6.91	6.29
OA29-II	243.67	267.67	220.00	7.61	8.36	6.87
Check	51.00	77.33	72.33	1.59	2.42	2.26

* Value of z for lines exceeds the one per cent point.

† Minimum significant difference between two means for 32 plants, 34.8 units.

‡ Minimum significant difference between two means for one plant, 1.087 units.

The data in Table 12 and in Figure 5B show small but significant differences between some of these lines. Without delineating the differences between the various lines, which can be more easily seen by examining Figure 5B, it can be shown that, on the basis of the differences found on Rustler and Golden Bantam, three pathogenic groups can be distinguished. These are represented by (a) OA39-I, (b)

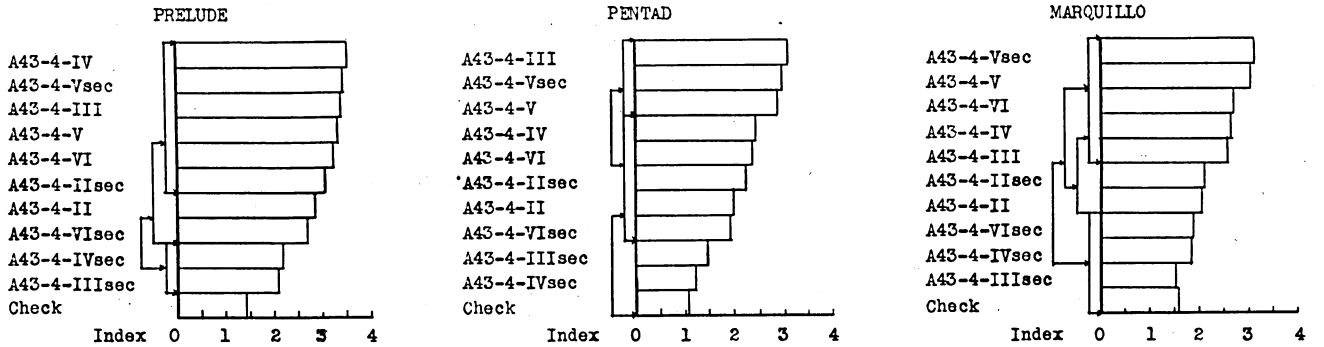

Fig. 6A. Relative pathogenicity on three varieties of wheat of five single ascospore isolates of *Gibberella saubinetii* and a variant from each. Index on single plant basis. Minimum significant difference between lines—0.534 unit.

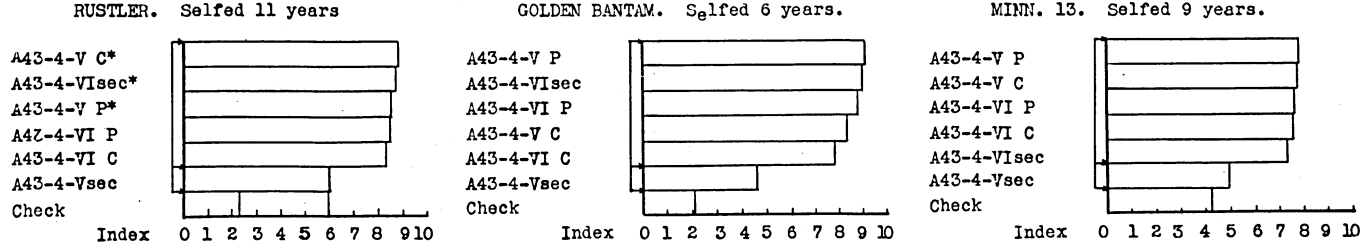

Fig. 6B. Relative pathogenicity on three selfed lines of corn of two ascospore isolates of *Gibberella saubinetii* grown on two different media and a variant from each. Index on single plant basis. Minimum significant difference between lines—1.30 units. * P—cultures grown on potato-dextrose agar. C—cultures grown on Coons' agar. sec—variants from cultures on Coons' agar.

OA29-II, and (c) OA34-I and OA32-I. Lines OA30-IV and OA12-V are not differentiated from the others. This experiment shows also that there is no correlation between pathogenicity and color intensity.

3. The effect of the cultural medium on pathogenicity.

The fact has been noted that the cultural appearance of *Gibberella saubinetii* on potato-dextrose agar differs from that on Coons' agar. It has also been seen that considerable variation took place on the latter medium.

The following experiment was designed to find if a change in pathogenicity accompanied a change in cultural appearance and to determine if the medium used had any cumulative effect on pathogenicity when such changes were not accompanied by a change in appearance.

The lines used were two single ascospore isolates from the same ascus and a variant of each. The parent cultures had been grown for ten weeks on both Coons' and potato-dextrose agar. The variants, lines A43-4-Vsec and A43-4-VIsec, arose as sectors in the cultures on Coons' agar. A43-4-VIsec was a perithecial type which has been previously described, while A43-4-Vsec was of the mottled type designated as C in Table 5.

The results of this experiment, given in Table 13 and Figure 6B, are rather distinct. It will be seen that line A43-4-Vsec is distinctly different from the other five on all three lines of corn, whereas these five are not significantly different from one another on any of the three lines of corn. This indicates that the medium upon which these lines had been grown did not affect the pathogenicity except where visible variation occurred. Even then one of these variants did not appear different from the parent lines.

Table 13

Index of Pathogenicity on Three Selfed Lines of Corn of Two Single Ascospore lines of *Gibberella saubinetii* Grown on Two Different Media and a Variant from Each Grown on Coons' Agar

Line and culture medium*	Mean index for 32 plants†			Mean index for 1 plant‡		
	Rustler	Gold. Bant.	Minn. 13	Rustler	Gold. Bant.	Minn. 13
A43-4-VI Coons'	268.33	251.33	241.00	8.39	7.85	7.53
A43-4-VI P.D.A.....	275.00	284.33	243.00	8.59	8.89	7.59
A43-4-VIsec§	279.00	287.67	236.00	8.72	8.99	7.37
A43-4-V Coons'.....	283.33	266.67	247.33	8.85	8.33	7.73
A43-4-V P.D.A.....	275.33	289.33	248.33	8.60	9.04	7.76
A43-4-Vsec§	191.67	149.67	156.67	5.99	4.68	4.90
Check	73.33	66.00	134.33	2.29	2.06	4.20

* Value of z for lines exceeds the one per cent point.

† Minimum significant difference between two means for 32 plants, 41.61 units.

‡ Minimum significant difference between two means for one plant, 1.30 units.

§ Grown on Coons' agar.

4. Differences in pathogenicity between parents and variants.

A more extensive experiment was made on wheat to determine the effect of visible cultural variation on pathogenicity. The parent lines used all arose as single ascospore isolates from one ascus and the variants as sectors on Coons' agar. Pictures of four of these parent lines and variants are shown on Plate VI.

The cultural type of the variant lines was as follows: A43-4-II sec, pink, slightly mottled, type G (see Table 5); A43-4-IIIsec, deep purple, type J; A43-4-IVsec, mottled with red border, type H; and A43-4-VIsec, perithecial, type K. Line A43-4-Vsec was not culturally different from its parent as it was in experiment 3. The culture used in the present test was reisolated from the corn in experiment 3 and apparently had reverted to its original type. As such reversion was uncommon in culture, this phenomenon suggests the interesting possibility that a short pathogenic existence is able to cause such a reversion. If this is true it would explain in part why so few distinctly different types are found when isolations are made from field material. It is also possible that the cultures became mixed during the process of isolation.

The results of this test are given in Table 14 and Figure 6A. The table also includes five perithecial lines which will be discussed in the following experiment. They were included in the same table because they were tested at the same time and analyzed as part of the same experiment.

Table 14

Index of Pathogencity on Three Varieties of Wheat of Single Ascospore Isolates and Variants of *Gibberella saubinetii*

Line*	Mean index for 25 plants†			Mean index for 1 plant‡		
	Prelude	Pentad	Marquillo	Prelude	Pentad	Marquillo
A43-4-II	70.75	48.75	51.25	2.83	1.95	2.05
A43-4-IIsec	77.00	56.00	54.00	3.08	2.24	2.16
A43-4-III	85.25	76.75	64.00	3.41	3.07	2.56
A43-4-IIIsec	52.75	36.00	37.75	2.11	1.44	1.51
A43-4-IV	87.75	60.25	65.75	3.51	2.41	2.63
A43-4-IVsec	54.75	30.75	46.00	2.19	1.23	1.84
A43-4-V	84.25	71.25	76.00	3.37	2.85	3.04
A43-4-Vsec	87.25	74.75	77.00	3.49	2.99	3.08
A43-4-VI	81.25	59.50	66.75	3.25	2.38	2.67
A43-4-VIsec	68.00	47.00	46.25	2.72	1.88	1.85
A43-4-I-I	47.00	30.25	44.75	1.88	1.21	1.79
A36-1-Vsec	77.75	53.75	57.75	3.11	2.15	2.31
A36-1-VIIIsec	57.00	34.25	47.25	2.28	1.37	1.89
OA39-III	56.00	42.75	40.75	2.24	1.71	1.63
9	79.25	64.00	62.50	3.17	2.56	2.50
Check	36.75	26.25	40.00	1.47	1.05	1.60

* Value of z for lines exceeds the one per cent point.

† Minimum significant difference between two means for 25 plants, 13.3 units.

† Minimum significant difference between two means for one plant, 0.534 unit.

No attempt will be made to separate the pathogenically different groups. It is sufficient to point out that lines A43-4-IIIsec and A43-4-IVsec are significantly less pathogenic than their respective parents on all three varieties of wheat and that A43-4-VIsec is less pathogenic than its parent line on Marquillo only. It will also be seen that lines A43-4-IIsec and A43-4-Vsec are not significantly different from their respective parents on any of the three varieties. Line A43-4-Vsec should probably be disregarded in this experiment, due to the possibility of improper identity mentioned above. If reversion did take place, it seems that it reverted with respect to pathogenicity as well as cultural appearance.

The results on lines A43-4-II, A43-4-III, and A43-4-IV are corroborated by the results of a preliminary experiment as shown in Table 15. These results were not analyzed, but it can be seen that differences similar to those in Table 14 exist here also. Plate VII is a picture showing the differences in pathogencity between four parent cultures and four variants on Prelude.

Table 15

Index of Pathogenicity on Three Varieties of Wheat of Three Single Ascospore Isolates of *Gibberella saubinetii* and a Variant from Each

Line	Mean index for 25 plants		
	Prelude	Pentad	Marquillo
A43-4-II	81.50 ± 4.07	81.50 ± 2.10	75.75 ± 4.54
A43-4-IIsec	92.25 ± 6.55	79.00 ± 4.19	70.75 ± 3.87
A43-4-III	87.50 ± 3.14	87.00 ± 4.61	71.25 ± 4.84
A43-4-IIIsec	47.75 ± 3.37	39.75 ± 2.59	41.00 ± 10.02
A43-4-IV	84.25 ± 4.39	74.75 ± 4.45	68.75 ± 4.92
A43-4-IVsec	60.25 ± 5.92	35.25 ± 2.24	42.25 ± 3.39
Check	37.75 ± 2.87	35.25 ± 4.25	30.25 ± 2.14

5. Pathogenic differences between perithecial lines.

A description of these lines has been given previously. It was shown that they were distinctly different in cultural appearance.

The data from pathogenicity tests on corn are presented in Table 16 and Figure 7A. The analysis of variance of this experiment is given in Table 17. The same method of analysis presented here was used in all of the seedling blight tests except that shown in Table 15.

The data in Table 16 reveal rather large differences between some of the lines, as well as smaller tho statistically significant ones. Lines A36-1-Vsec and 9 were not significantly different on any of the three lines of corn. It will be remembered that they differed in culture only in the color of the aerial mycelium. By comparing the reaction on corn

Table 16

Index of Pathogenicity on Three Selfed Lines of Corn of Six Perithecial Lines of *Gibberella saubinetii*

Line	Mean index for 32 plants*									Mean index for 1 plant (Mean of 2 experiments)†		
	Rustler			Golden Bantam			Minn. 13					
	1st exp.	2nd exp.	Mean 2 exps.	1st exp.	2nd exp.	Mean 2 exps.	1st exp.	2nd exp.	Mean 2 exps.	Rustler	Golden Bantam	Minn. 13
A43-4-VIsec	277.33	272.33	274.82	271.67	281.00	276.33	230.67	237.67	234.17	8.59	8.64	7.32
A43-4-I-I	145.33	206.00	175.66	121.33	108.67	115.00	145.00	131.00	138.00	5.49	3.59	4.31
A36-1-Vsec	281.67	283.00	282.33	284.67	287.00	285.83	273.67	282.00	277.83	8.82	8.93	8.68
A36-1-VIIIsec	208.33	200.67	204.50	166.67	132.33	149.50	156.67	151.67	154.17	6.39	4.67	4.82
OA39-III	179.33	211.00	195.16	192.33	162.00	177.16	184.00	172.67	178.33	6.10	5.54	5.57
9	282.33	282.33	282.33	294.67	289.00	291.83	266.67	258.67	262.67	8.82	9.12	8.21
Check	77.67	61.67	69.67	69.33	73.33	71.33	75.33	89.67	82.50	2.18	2.23	2.58

* Minimum significant difference between two means for 32 plants at the 1 per cent point = 25.29 units.

† Minimum significant difference between two means on a single plant basis at the 1 per cent point = $\frac{25.29}{32}$ = 0.790 unit.

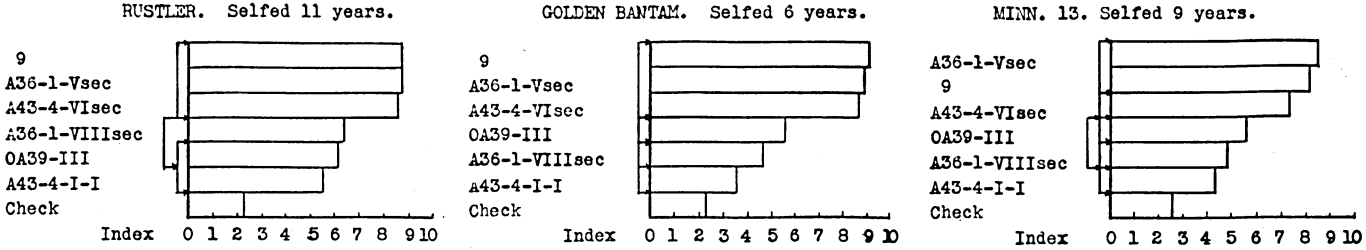

Fig. 7A. Relative pathogenicity on three selfed lines of corn of six perithecial lines of *Gibberella saubinetii*. Index on single plant basis. Minimum significant difference between lines—0.790 unit.

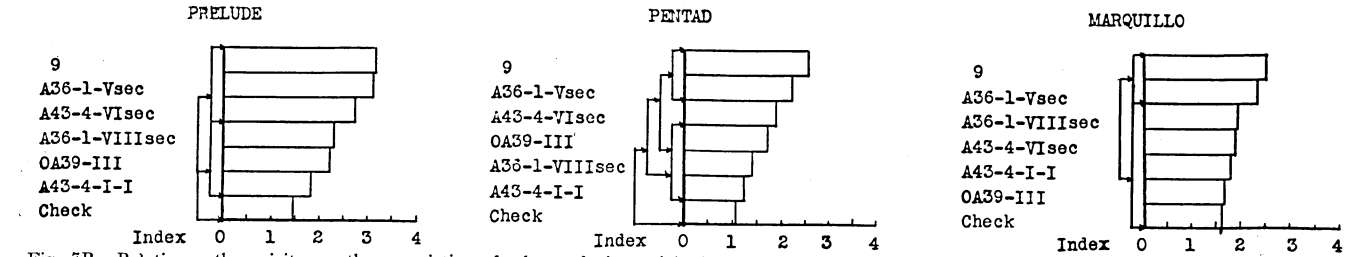

Fig. 7B. Relative pathogenicity on three varieties of wheat of six perithecial lines of *Gibberella saubinetii*. Index on single plant basis. Minimum significant difference between lines—0.534 unit.

it can be seen that five pathogenically different groups are present. These are (a) A43-4-I-I, (b) A36-1-VIIIsec, and (c) OA39-III, all of which are significantly different on Golden Bantam; (d) A43-4-VI sec, which is significantly different from all others on Minnesota 13; and (e) 9 and A36-1-Vsec, which are not significantly different from each other on any of the three lines of corn, but which are significantly different from all others on Minnesota 13. (See Fig. 8.)

Table 17

Analysis of Variance of the Indices of Pathogenicity on Three Selfed Lines of Corn of Six Perithecial Lines of *Gibberella saubinetii*

Variation due to	Sums of squares	Degrees of freedom	Variance	*Z*
Replications	1,481.72	2	740.86	.....
Varieties	11,399.48	2	5,699.74	1.5144*
Lines	665,672.32	6	110,945.39	2.9987*
Experiments	7.63	1	7.63	.....
Interaction				
Varieties x lines.....	22,510.64	12	1,815.89	0.9585*
Varieties x experiments..	1,866.21	2	933.10	0.6089†
Lines x experiments.....	1,950.76	6	325.12	0.0828
Varieties x lines x exp'ts.	8,358.56	12	696.55	0.4641*
Error	22,609.62	82	275.73	.....
Total	735,856.94	125	.....	.....

* Value of z exceeds the 1 per cent point.
† Value of z exceeds the 5 per cent point.

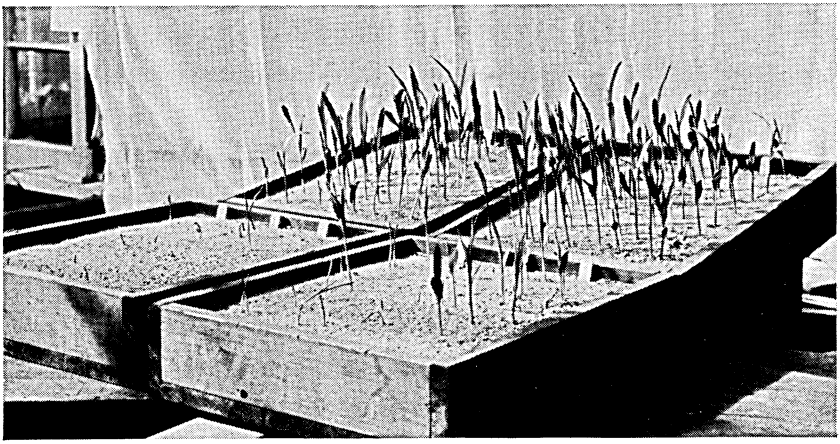

Fig. 8. Relative pathogenicity of three perithecia forming lines of *Gibberella saubinetii* on corn. Three selfed lines of corn are planted in each flat.
Upper row: left, line A43-4-I-I; right, check (uninoculated).
Lower row: left, line 9; right, line OA39-III.

The results of the experiments on wheat are given in Table 14 and Figure 7B. The differences between these cultures in their pathogenicity on wheat are not so great as they were on corn. Only two

groups differing in pathogenicity can be made, consisting of (a) lines 9 and A36-1-Vsec and (b) lines A36-1-VIIIsec, OA39-III, and A43-4-I-I. A43-4-VIsec was not differentiated from either of these groups.

This experiment shows, as do experiments 3 and 4, that different cultural types of *Gibberella saubinetii* may also show rather wide differences in pathogenicity. It should be remembered that all of these lines, except line 9, arose as variants in culture.

The Correlation of Rate of Growth and Pathogenicity

Figure 9A shows graphically the indices of pathogenicity of the six perithecial lines on corn and also the diameters of four-day-old colonies of the same lines when grown at 17° C. on two media. This was the temperature at which the corn seedling blight experiments were run. It indicates that there is some correlation between pathogenicity and rate of growth, especially on potato-dextrose agar. The differences between individual lines is not so great with respect to rate of growth as to pathogenicity. There is also a reversal of this relationship when lines A36-1-VIIIsec and OA39-III are compared. Here line OA39-III is more pathogenic on two lines of corn than A36-1-VIIIsec, but grows more slowly.

A comparison of diameters of four-day-old colonies grown at 24° C. on Coons' and potato-dextrose agar and their relative pathogenicity on wheat is given graphically in Figure 9B. Here again the relationship between pathogenicity and rate of growth on potato-dextrose agar seems to be fairly close for all cultures except OA39-III. This culture seemed to grow more slowly compared to its pathogenicity than did the other cultures.

Discussion of Seedling Blight Experiments

The problem of drawing conclusions from seedling blight studies involves certain considerations which should be discussed before attempting to evaluate the experiments which have been described.

An examination of Figures 5, 6, and 7 reveals that there are statistically significant differences between lines and groups of lines in each experiment. Some of these differences are great enough to be visible by an examination of either the experimental material itself or the unanalyzed data compiled therefrom. Others are not apparent until the data have been analyzed.

It is the significance of the latter differences which should be regarded with caution. Unless some undetected error has been made in the system of taking notes, those lines differing by small, but significant, amounts must be regarded as truly different at that time. Further

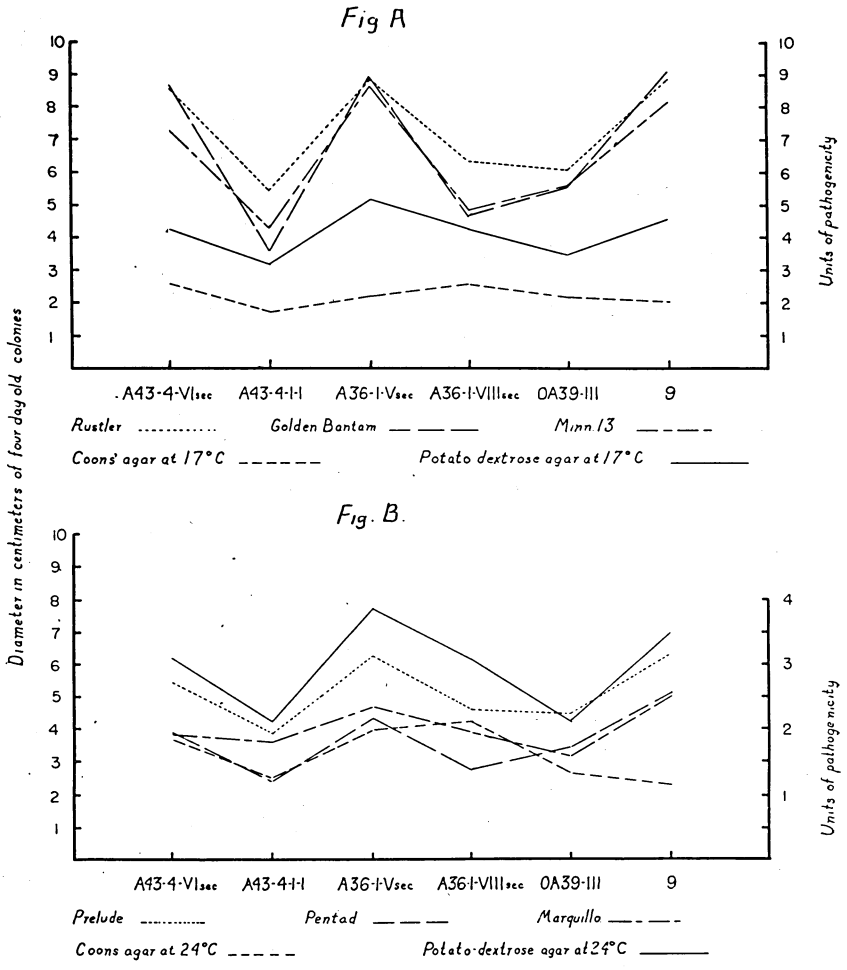

Fig. 9. A. Graph showing the relationship between the pathogenicity on corn of six perithecia-producing lines of *Gibberella saubinetii* and the growth rates of the same lines at 17° C.

B. Graph showing the relationship between the pathogenicity on wheat of six perithecia-producing lines of *Gibberella saubinetii* and the growth rates of the same lines at 24° C.

repetitions of these experiments are desirable to determine whether the differences between lines remain constant in the absence of visible variations in culture. A single experiment has been described which indicates that the type of medium upon which the line has grown produces no significant differences in pathogenicity so long as it remains visibly stable in culture. On the other hand, Leonian (34) states that *Fusarium moniliforme* is as unstable pathogenically as it is culturally. His

pathogenicity studies were not controlled so carefully as the experiments here described, and he does not state whether or not variations in pathogenicity are accompanied by changes in cultural appearance.

Should it be possible to show that such small differences are consistent, the problem of demonstrating whether or not two given lines are different would demand that a large number of different host varieties and lines be inoculated. This is apparent from the fact that the limited data here presented indicate that two lines may be different on one line of corn or variety of wheat, but not on another. The list of host plants should include other cereals beside wheat and corn, since the fungus is able to produce seedling blight on a number of such crops.

Given such a large number of hosts, it is possible that enormous numbers of significantly different lines could be recognized. It is also possible that the host plants would fall into relatively few groups of similar susceptibility, such as Stakman and Levine (50) found in selecting differential hosts for the determination of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn.

The two problems are not strictly comparable, however, since in the case of stem rust the phenomena of resistance and susceptibility are fairly well defined over a rather wide range of environmental conditions and can be recognized by qualitative differences in symptoms. In the case of seedling blight the amount of injury caused is very dependent on environmental conditions, and the measure of damage used is quantitative rather than qualitative.

It can be seen, therefore, that the separation of pathogenic strains of *Gibberella saubinetii* or any other root-rotting organism is fraught with great difficulties. Since the method used in these studies has not been sufficiently tested and still has certain shortcomings, these things must be kept in mind in drawing conclusions for the experiments described.

A survey of the literature reveals that pathogenic strains of *Fusaria* have been described by numerous workers. Among the wilt-producing *Fusaria* such strains have been described by Broadfoot (6) in *Fusarium lini* Bolley on flax, by Fahmy (21) in *F. vasinfectum* Atk. on cotton, by White (56) in *F. lycopersici* Sacc. on tomato, by Snyder (47) in *F. orthoceras* App. and Wr. var. *pisi* Linford on peas, and by Sleeth (45) in *F. niveum* E.F.S. on watermelon. Leach (32) described an organism similar to *F. niveum* which caused a wilt of muskmelon. This strain would not cause wilt of watermelons, whereas a watermelon strain of *F. niveum* wilted watermelons but not muskmelons. Nisikado (39) has demonstrated that physiologic specialization occurs

in *F. lateritium* Nees. and *F. oxysporum* Schlecht. which cause rotting of ripe fruit.

Among the *Fusaria* attacking cereals, Tu (54) reported pathogenic strains of *Fusarium culmorum* (W.G.Sm.) Sacc., *F. avenaceum* (Fr.) Sacc., and *Gibberella saubinetii* which differed in their ability to cause head blight of cereals. Greaney and Bailey (23) isolated four cultures of a *Fusarium* which they identified tentatively as *F. graminearum*. These strains fell into two groups differing in ability to cause seedling blight of wheat. Pathogenic differences among isolates of *F. moniliforme* have been demonstrated by Leonian (34) and Henry (25). On the other hand, Peuser (40) found no biologic specialization among thirty-seven isolates of *Calonectria graminicola* (Berk. and Br.) Wr. (*F. nivale* Caes.).

Pathogenic strains have been described in other species of soil organisms which cause seedling blights and root rots. Some of the more important of these contributions are those of Christensen (12), who found numerous parasitic strains of *Helminthosporium sativum* Pam., King, and Bak., and Henry (25), who found parasitic differences among isolates of an unidentified *Helminthosporium*. This list might be extended to include several other soil fungi.

The results of the present studies of the pathogenicity of *Gibberella saubinetii* furnish evidence on two questions. In the first place they indicate that lines of the fungus originating as single ascospores isolated from naturally occurring material differ but little in pathogenicity.

It is true that small but statistically significant differences were found. However, much more work needs to be done to show whether or not these differences are consistent and whether or not they represent true genetic differences or semi-permanent variations of the Dauermodifikationen type described by Jollos (29) in *Paramecium*.

The present studies were made with two few cultures to prove conclusively that great differences in pathogenicity do not occur in the fungus in nature. However, it is interesting to note that a single cultural type predominated among the isolates, and that those tested did not differ a great deal in pathogenicity. This agrees with the conclusions of Peuser (40), who failed to find biologic specialization in isolates of *Calonectria graminicola*.

These studies show further that cultural variants in *G. saubinetii* may lose their pathogenicity and that different types of the fungus which arise in culture may differ decidedly in virulence on corn and wheat seedlings as well as in ability to cause head blight of wheat and barley. These results are in harmony with the observations of many workers who have dealt with parasitic fungi in culture.

Here again the question of the stability of such types with respect to pathogenicity has not been proven. Many of the variants studied were relatively stable in cultural appearance after variation had occurred, and it is possible that the same is true of relatively great differences in pathogenicity.

The significance of the present work lies in the fact that it shows that *G. saubinetii,* like a great majority of fungi, varies in culture, and that with these cultural changes, variation in pathogenicity may also occur.

It is felt that these studies also show that methods can be used which will reveal small differences in pathogenicity, thus affording a tool which may be used in further studies of the variability of the organism.

DISCUSSION AND CONCLUSIONS

It seems inevitable that a research problem dealing with species of *Fusarium* should become involved in a study of variability. Investigators who have overlooked or disregarded this phase of the problem have been subject to criticism on this account. The work of numerous investigators leaves little doubt that variation is one of the outstanding characteristics of the genus, and Leonian (33) makes the statement that the only constant thing in *Fusarium* is its inconstancy.

Altho variation in this genus is often observed in culture, the extent of the occurrence of variants or physiologic strains in nature has not been so well demonstrated. This is due to the fact that most *Fusaria* must be grown for long periods in culture before their specific identity can be accurately known. During this time variation may and usually does occur, and unless an accurate record is kept of the original characteristics of the isolates, their value in determining the occurrence in nature of physiologic strains is lost.

It is true that if one isolates wilt-producing *Fusaria* from diseased host plants he can be reasonably sure that he has the pathogen. Descriptions of parasitic differences between such isolates have been made by Snyder (47) and others, but even then it does not seem that one can be entirely sure of the specific identity of the organisms unless subsequent specific determinations are made by approved methods. The value of such work is not lost through a failure to do so, however, because the mere demonstration of the range of parasitism among a group of isolates producing a disease is valuable, even if their specific identity is not known with certainty. For practical purposes it seems that the plant pathologist can afford to be less particular about the specific identity of *Fusaria* as long as he knows the range of parasitism of the organisms causing a definite disease.

The problem of determining the range of parasitic capabilities in a given species of *Fusarium* causing seedling blight is even more complicated, because here the identity of the original pathogen may be confused with various saprophytes in the same genus.

In the present studies, the existence of a perfect stage made possible a survey of the cultural characteristics of the fungus as it was first isolated from diseased host plants, with assurance of the specific identity of the fungus.

The results of this survey, which shows most of the cultures to be of a similar cultural type, are more or less striking in view of the fact that many plant pathogens comprise a number of cultural strains when first isolated. It is admitted that a larger number of types might have been distinguished on a larger number of differential media. A limited number of pathogenicity experiments revealed no striking differences between isolates of similar cultural appearance. However, these tests are too few in number to be conclusive.

The nature of cultural variants in *Fusarium,* as well as in other fungi, is a matter of considerable controversy. Appel and Wollenweber (2), besides recognizing the effect of the culture medium upon growth characters, found that their cultures changed from a mycelial type producing very few conidia of irregular size and septation to types which formed abundant conidia of a relatively high degree of morphological uniformity. This change they thought was brought about by the use of conidia rather than mycelium in transferring the cultures from one medium to another. The former type they designated as "Ankultur" and the latter as "Hochkultur." After being held for some time in culture, they found that the fungus might change again to a type producing small, few-septate spores. This they considered was a degeneration form which they designated as "Abkultur."

Brown (8) challenges this interpretation and gives evidence which indicates that Appel and Wollenweber, by selecting conidia as inocula, were in reality selecting small variants characterized by the production of abundant conidia. Brown states further that in the absence of saltation there is no difference in the relative potency of mycelial or conidial inocula. He also shows that variants from one species of *Fusarium* resembled another supposedly distinct species more closely than it did the parent. Wollenweber (59), in his most recent work on *Fusaria,* has classified both of the species which Brown investigated as varieties of the same species.

The possibility that cultural variants in fungi may be of the same type as the "Dauermodifikationen" described by Jollos (29) is mentioned by Caldis and Coons (10). This viewpoint is also discussed

by Stakman, Christensen, Eide, and Peturson (49). Caldis and Coons (10) consider the variations which they observed to be similar to "Dauermodifikationen" and state that they resemble the "Abkulturen" described by Appel and Wollenweber (2) or the so-called attenuated cultures of bacteria and fungi.

Brown (7) prefers to use the term "saltation" to designate definite spontaneous variations which are more or less stable. Dickinson (17), on the other hand, states definitely that variations in *Fusarium fructigenum* are true mutations, as his investigations failed to give any evidence of cytoplasmic inheritance.

If the variations in *Gibberella saubinetii* are Dauermodifikationen, or if they are due to some type of cytoplasmic change, it seems reasonable to suspect that these variations would disappear when the fungus is reproduced sexually. It also seems reasonable to assume that if the variants represent stages in the life cycle, as suggested by Leonian (33), they would also disappear in the process of sexual reproduction, as this in itself is a definite, recognized stage in the life cycle. This criterion has been applied by Stakman, Christensen, Eide, and Peturson (49), who found that certain variants in *Ustilago zeae* (Beckm.) Ung. persisted through the sexual stage and could therefore be regarded as true mutants, or at least resulting from genotypic changes.

The evidence in the present investigation, while as yet incomplete, indicates that at least some of the variations in *Gibberella saubinetii* persist through the sexual stage. Lines A43-4-VIsec and A36-1-VIII sec are easily distinguishable from the parental type, even disregarding the ability to produce perithecia in culture. It is the opinion of the author that even this character should be regarded as a permanent variation from the parental type. If it were not, one would expect that isolations of ascospores formed in culture would again yield the parental type; i.e., one which does not produce perithecia and ascospores. The parent lines, it will be remembered, all originated as single ascospores from field material, and yet did not produce perithecia in culture until a variant with this characteristic appeared. From this one may conclude that these variants at least are not "Dauermodifikationen," nor stages in a life cycle, but rather are due to permanent changes in the genotype of the fungus. Since most of the variants observed apparently lost the ability to form perithecia, even under conditions approximating those found in nature, it was not possible to investigate the inheritance of the variant characters.

It is apparent from the review of literature given in the section on perithecial formation that *Gibberella saubinetii* is as unstable in this respect as it is with respect to other cultural characters. The fact

that the fungus will produce perithecia in the field but not in the laboratory is easily explained by the wide differences between field and laboratory conditions with respect to nutrition and environmental factors. But the spontaneous appearance of lines with the ability to form perithecia on a highly artificial medium indicates that this property is dependent upon factors other than simply the presence of the necessary sex factors. The present investigations show that this character arises in a manner similar to the way in which other cultural variations occur and should be regarded as such.

The experiments which show that *Gibberella saubinetii* is homothallic are not of any particular significance except as they establish the fact itself. This does not preclude the possibility of mating different strains of the fungus, for altho little is known of the nuclear phenomena in this and other homothallic ascomycetes, it is known that hyphal fusions are common and combinations of nuclei of different strains may take place in this way. The presence of lines which produce rudimentary perithecia also offers the possibility that by mating them mature ascospores will be formed. Investigations along these lines have been planned.

The work on pathogenicity has been carried but little beyond the development of a method suitable for testing the pathogenicity of an organism so sensitive to environmental influences as is *Gibberella saubinetii*. It is felt that this in itself constitutes a definite contribution. The limited tests made indicate that there is little difference in pathogenicity between cultures of the fungus when it is first isolated, this observation agreeing with the fact that most of the original isolates differed but little in cultural type. The pathogenicity studies show further that variations in pathogenicity as well as in appearance occur in culture.

SUMMARY

1. A number of isolations were made of naturally occurring ascospores of *Gibberella saubinetii*. All but 31 of 325 such isolates were of the same cultural type, varying only in degree of color intensity. Of the 31, 24 were like the majority on potato-dextrose agar, but differed on Coons' agar. The remaining seven comprised three types, two represented by one culture each, and the third by five cultures from a single collection.

2. Variation in appearance in *Gibberella saubinetii* is common on Coons' agar but less frequent on potato-dextrose agar. Most of the variants fell into relatively few cultural groups.

3. When first isolated from field material, the single ascospore cultures, with one exception, did not produce perithecia in culture. In

the absence of visible variation, most of these cultures are able to form perithecia in the greenhouse under conditions approximating those in the field. The variants apparently lost their ability to form perithecia under these conditions.

4. Attempts were made to stimulate perithecial production by growing the fungi on different media, by subjecting them to different temperatures, and by exposing them to ultra-violet light. All of these attempts resulted in failure.

5. The ability to produce perithecia in culture may appear spontaneously, in the manner that other cultural variations appear. Three such variants were isolated.

6. Perithecial variants retain their cultural characteristics and ability to produce perithecia when propagated from single ascospores, conidia, or germ tubes from individual cells of either spore type. This shows that the cultures investigated are homothallic. Three cultures which formed rudimentary perithecia were never found to produce ascospores.

7. The formation of ascospores in the ascus takes place in a manner common to most ascomycetes. The ascospores are formed from a single nucleus, but consist of four cells when mature. Each cell usually contains a single nucleus, altho a few cells were observed which contained two.

8. The cells of the conidia are also predominantly uninucleate, altho some may contain two, four, or even six nuclei.

9. A method was developed for determining the pathogenicity of *Gibberella saubinetii* on corn and wheat seedlings. By this method it is possible to detect very small differences in virulence of different cultures.

10. In the absence of variation, ascospore isolates from field material were not found to differ greatly in pathogenicity. Cultural variants may differ decidedly in pathogenicity, some of these being less virulent than the parent culture.

LITERATURE CITED

1. Adams, J. F. Observations on wheat scab in Pennsylvania and its pathological histology. Phytopath. **11**:115-124. 1921.
2. Appel, O., and H. W. Wollenweber. Grundlagen einer Monographie der Gattung Fusarium (Link.). Arb. aus der Kaiserlichen Biol. Inst. für Land- und Forstw. **8**:1-198. 1910.
3. Atanasoff, Dimitr. Fusarium-blight (scab) of wheat and cereals. Jour. Agr. Res. **20**:1-32. 1920.
4. ———— Fusarium blight of the cereal crops. Medel. Landbouwhoogeschool, Wageningen. **27**:1-132. 1923.

5. Bennett, F. T. *Gibberella saubinetii* (Mont.) Sacc. on British cereals. II. Physiological and pathological studies. Ann. Appl. Biol. **18**:158-177. 1931.
6. Broadfoot, W. C. Studies on the parasitism of *Fusarium lini* Bolley. Phytopath. **16**:951-978. 1926.
7. Brown, W. Studies on the genus Fusarium. IV. On the occurrence of saltations. Ann. Bot. **40**:223-243. 1926.
8. ———— Studies on the genus Fusarium. VI. General description of strains, together with a discussion of the principles at present adopted in the classification of Fusarium. Ann. Bot. **42**:285-304. 1928.
9. Buisman, Christine. Uber die Biologie und den Parasitismus der Gattung Ceratostomella Sacc. Phytopath. Ztschr. **6**:429-439. 1933.
10. Caldis, P. D., and G. H. Coons. Achromatic variations in pathogenic fungi. Mich. Acad. Sci., Arts and Letters. **6**:191-236. 1926.
11. Chester, F. D. The scab of wheat. Del. Agr. Exp. Sta. Rept. **3**:89-90. 1890.
12. Christensen, J. J. Physiologic specialization and parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Bul. 37. 1926.
13. ———— Studies on the genetics of *Ustilago zeae*. Phytopath. Ztschr. **4**:129-188. 1931.
14. ————, E. C. Stakman, and F. R. Immer. Susceptibility of wheat varieties and hybrids to Fusarial head blight in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 59. 1929.
15. Coons, G. H., and M. C. Strong. The diagnosis of species of Fusarium by use of growth-inhibiting substances in the culture medium. Mich. Agr. Exp. Sta. Tech. Bul. 115. 1931.
16. Dodge, B. O. Inheritance of the albinistic non-conidial characters in interspecific hybrids in Neurospora. Mycologia **23**:1-50. 1931.
17. Dickinson, Sydney. The nature of saltation in Fusarium and Helminthosporium. Minn. Agr. Exp. Sta. Tech. Bul. 88. 1932.
18. ———— The technique of isolation in microbiology. Phytopath. **23**: 357-367. 1933.
19. Dickson, James G. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetii*. Jour. Agr. Res. **23**:837-870. 1923.
20. ————, Helen Johann, and Grace O. Wineland. Second progress report on the Fusarium blight (scab) of wheat. (Abst.) Phytopath. **11**:35. 1921.
21. Fahmy, T. The Fusarium disease (wilt) of cotton and its control. Phytopath. **17**:749-767. 1927.
22. Fisher, R. A. Statistical methods for research workers. 304 pp. Oliver and Boyd, London. 1932.
23. Greaney, F. J., and D. L. Bailey. Root-rots and foot-rots of wheat in Manitoba. Canada Dept. of Agr., Div. of Bot., Bul. 85. 1927.
24. Hayes, H. K., I. J. Johnson, and E. C. Stakman. Reaction of maize seedlings to *Gibberella saubinetii*. Phytopath. **23**:905-911. 1933.
25. Henry, A. W. Root-rots of wheat. Minn. Agr. Exp. Sta. Tech. Bul. 22. 1924.
26. Hoffer, G. N., and J. R. Holbert. Selection of disease-free seed corn. Ind. Agr. Exp. Sta. Bul. 224. 1918.

27. ————, A. G. Johnson, and D. Atanasoff. Corn root-rot and wheat scab. Jour. Agr. Res. 14:611-612. 1918.
28. Hynes, H. J. On the occurrence in New South Wales of *Gibberella saubinetii,* the organism causing scab of wheat and other cereals. Jour. and Proc. Roy. Soc. of N.S.W. 5:337-348. 1924.
29. Jollos, Victor. Experimentelle Vererbungsstudien an Infusorien. Zeit. Ind. Abst. und Verer. 24:77-97. 1920.
30. Kasai, M. Cultural studies with *Gibberella saubinetii* (Mont.) Sacc. which is parasitic on rice plants. Ber. Ohara Inst. Landw. Forsch. 2:259-272. 1923.
31. Koehler, Benjamin, J. G. Dickson, and James R. Holbert. Wheat scab and corn root-rot caused by *Gibberella saubinetii* in relation to crop successions. Jour. Agr. Res. 27:861-879. 1924.
32. Leach, J. G. A destructive Fusarium wilt of muskmelons. Phytopath. 23: 554-556. 1933.
33. Leonian, Leon H. Studies on the variability and dissociation in the genus Fusarium. Phytopath. 19:753-868. 1929.
34. ———— The pathogenicity and variability of *Fusarium moniliforme* from corn. West Va. Agr. Exp. Sta. Bul. 248. 1932.
35. MacInnes, Jean, and Raymond Fogelman. Wheat scab in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 18. 1923.
36. Manns, Thomas F., and Claude E. Phillips. Corn root-rot studies. Jour. Agr. Res. 27:957-964. 1924.
37. McIndoe, K. G. The inheritance of the reaction of maize to *Gibberella saubinetii.* Phytopath. 21:615-637. 1931.
38. Mitter, J. H. Studies in the genus Fusarium VIII. Saltation in the section Discolor. Ann. Bot. 43:379-410. 1929.
39. Nisikado, Y. Beiträge zur physiologischen Spezialisierung einiger obstbewohnender Fusarien. Ber. Ohara Inst. fur Landw. Forsch. 5:107-144. 1931.
40. Peuser, H. Untersuchungen über das Vorkommen biologischer Rassen von *Fusarium nivale* Ces. Phytopath. Ztschr. 4:113-125. 1931.
41. Ridgway, Robert. Color standards and color nomenclature. Washington, D.C. 1912.
42. Selby, A. D. Some diseases of wheat and oats. Ohio Agr. Exp. Sta. Bul. 97. 1898.
43. ————, and T. F. Manns. Studies in diseases of cereals and grasses. Ohio. Agr. Exp. Sta. Bul. 203. 187-236. 1909.
44. Sherbakoff, C. D. Fusaria of potatoes. Univ. of Cornell Mem. 6. 1915.
45. Sleeth, Bailey. *Fusarium niveum,* the cause of watermelon wilt. W. Va. Agr. Exp. Sta. Bul. 257. 1934.
46. Smith, Worthington G. Diseases of field and garden crops. 209 pp. London, 1884.
47. Snyder, William C. Variability in the pea-wilt organism, *Fusarium orthoceras* var. *pisi.* Jour. Agr. Res. 47:65-88. 1933.
48. Sorokin, N. Über einige Krankheiten der Kulturpflanzen in Süd-Ussurischen Gebiete. Wissenschaftliche Arbeiten der Naturforschergesellschaft der Kais Universität zu Kazan. 22: (Russian). Abst. in Zeit. fur Pflanzenkr. 1:236-239. 1891.

49. Stakman, E. C., J. J. Christensen, C. J. Eide, and Bjorn Peturson. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Exp. Sta. Tech. Bul. 65. 1929.
50. ————, and M. N. Levine. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.
51. Stevens, F. L. Effects of ultra-violet irradiation on various fungi. Bot. Gaz. **86**:211-225. 1928.
52. ———— The effects of ultra-violet irradiation on various Ascomycetes, Sphaeropsidales and Hyphomycetes. Zentralblatt für Bakt. II, **82**: 164-174. 1930.
53. ———— The ascigerous stage of *Colletotrichum lagenarium* induced by ultra-violet irradiation. Mycologia **23**:134-139. 1931.
54. Tu, Chih. Physiologic specialization in *Fusarium* spp. causing head blight of small grains. Minn. Agr. Exp. Sta. Tech. Bul. 74. 1930.
55. Weed, C. M. The scab of wheat heads. (*Fusisporium culmorum*) Amer. Nat. **24**:958. 1890.
56. White, R. P. Studies on the tomato wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. **34**:197-239. 1927.
57. Wollenweber, H. W. Studies on the Fusarium problem. Phytopath. **3**: 24-50. 1913.
58. ———— Über *Fusarium roseum* Link. Ber. der. Deutsch. Bot. Ges. **35**: 743-745. 1917.
59. ———— Fusarium monographie. Fungi parasitici et saprophytici. Zeit. für Parasitenk. **3**:269-516. 1931.
60. Woronin, M. On the intoxicating bread in the South Usurian provinces. Bot. Zapiski. Imp. S. P. Univ. 1890. (Russian).

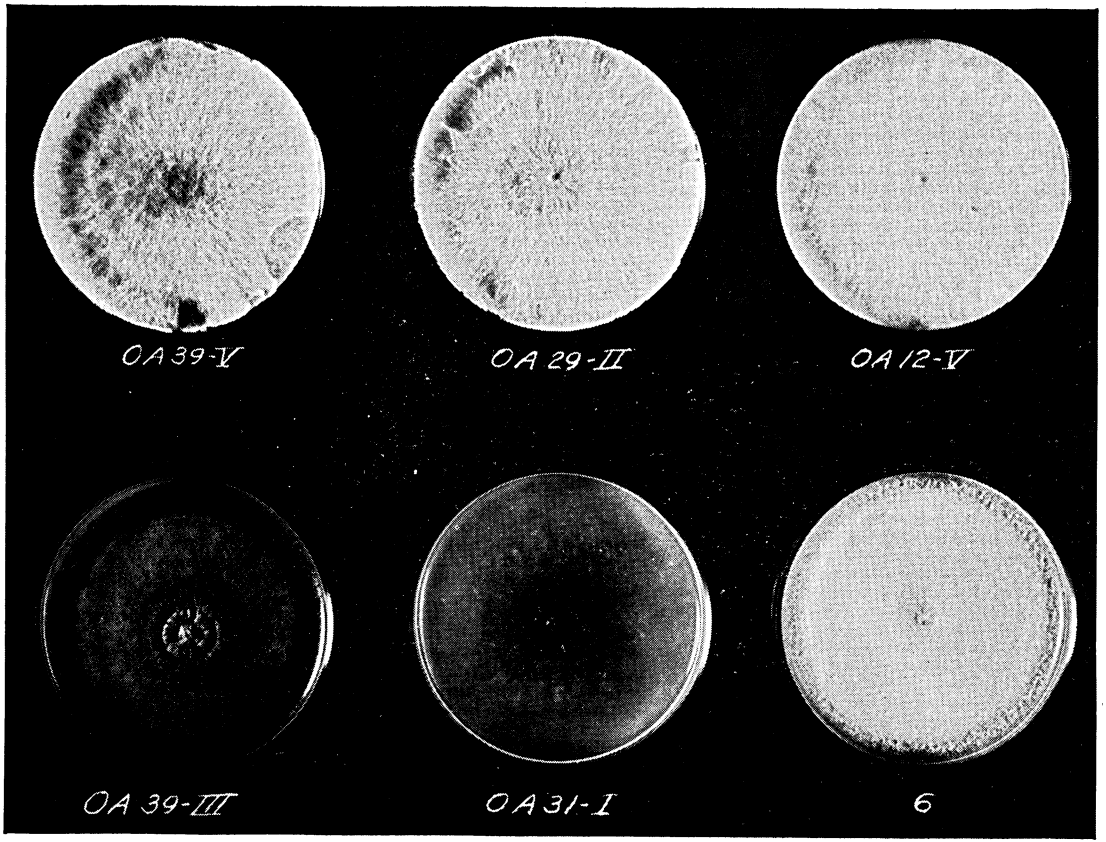
OA 39-V
OA 29-II
OA 12-V
OA 39-III
OA 31-I
6

Plate I

Cultural types of *Gibberella saubinetii* arising from naturally occuring ascospores. Top view of cultures.

OA39-V. Predominant type; Eugenia red.
OA29-II. Predominant type; pale pink.
OA12-V. Mottled type; identical with variant type C.
OA39-III. Type developing perithecia in culture.
OA31-I. Slow-growing type represented by isolates OA31-I to OA31-V, inclusive.
6. *Gibberella moniliformis*.

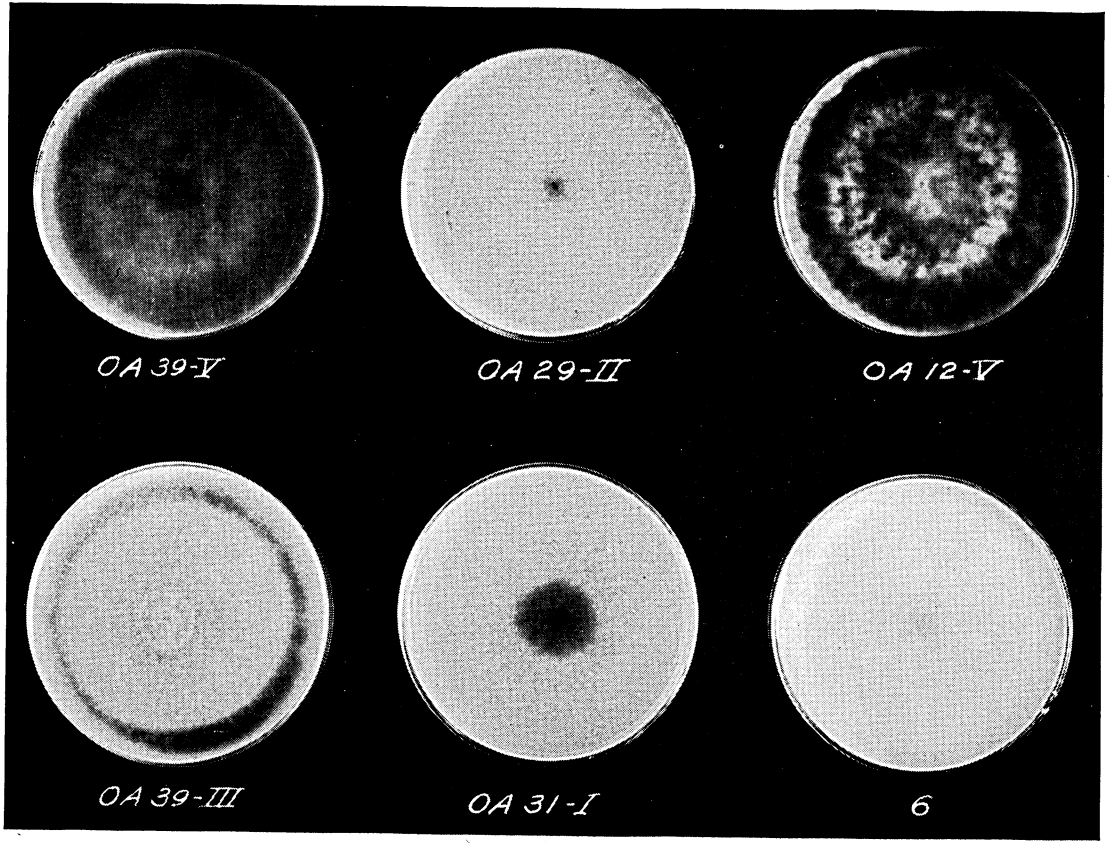
OA 39-V
OA 29-II
OA 12-V
OA 39-III
OA 31-I
6

Plate II

Cultural types of *Gibberella saubinetii* arising from naturally occurring ascospores. Bottom view of cultures.

OA39-V. Predominant type; Eugenia red.
OA29-II. Predominant type; pale pink.
OA12-V. Mottled type; identical with variant type C.
OA39-III. Type developing perithecia in culture.
OA31-I. Slow-growing type represented by isolates OA31-I to OA31-V, inclusive.
6. *Gibberella moniliformis.*

I
II
III
IV
V
VI
VII
VIII

Plate III

Eight single ascospore isolates from a single ascus of *Gibberella saubinetii*. Top view of cultures. A38-1 omitted in the labels on the plate.

A38-1-I. Type I. This line later lost the dark color shown in the photograph and became a uniform buff color.
A38-1-II. Normal. This line later became type H.
A38-1-III. Normal. This line later became type C.
A38-1-IV. This culture as shown here is an extreme example of type D. It later became more like A38-1-V, except that the border was darker.
A38-1-V. Type D.
A38-1-VI. Type C.
A38-1-VII. Normal.
A38-1-VIII. Normal; producing sectors.

I
II
III
IV
V
VI
VII
VIII

Plate IV

Eight single ascospore isolates from a single ascus of *Gibberella saubinetii*. Bottom view of cultures. A38-1 omitted in the labels on the plate.

A38-1-I. Type I. This line later lost the dark color shown in the photograph and became a uniform buff color.

A38-1-II. Normal. This line later became type H.

A38-1-III. Normal. This line later became type C.

A38-1-IV. This culture as shown here is an extreme example of type D. It later became more like A38-1-V, except that the border was darker.

A38-1-V. Type D.

A38-1-VI. Type C.

A38-1-VII. Normal.

A38-1-VIII. Normal; producing sectors.

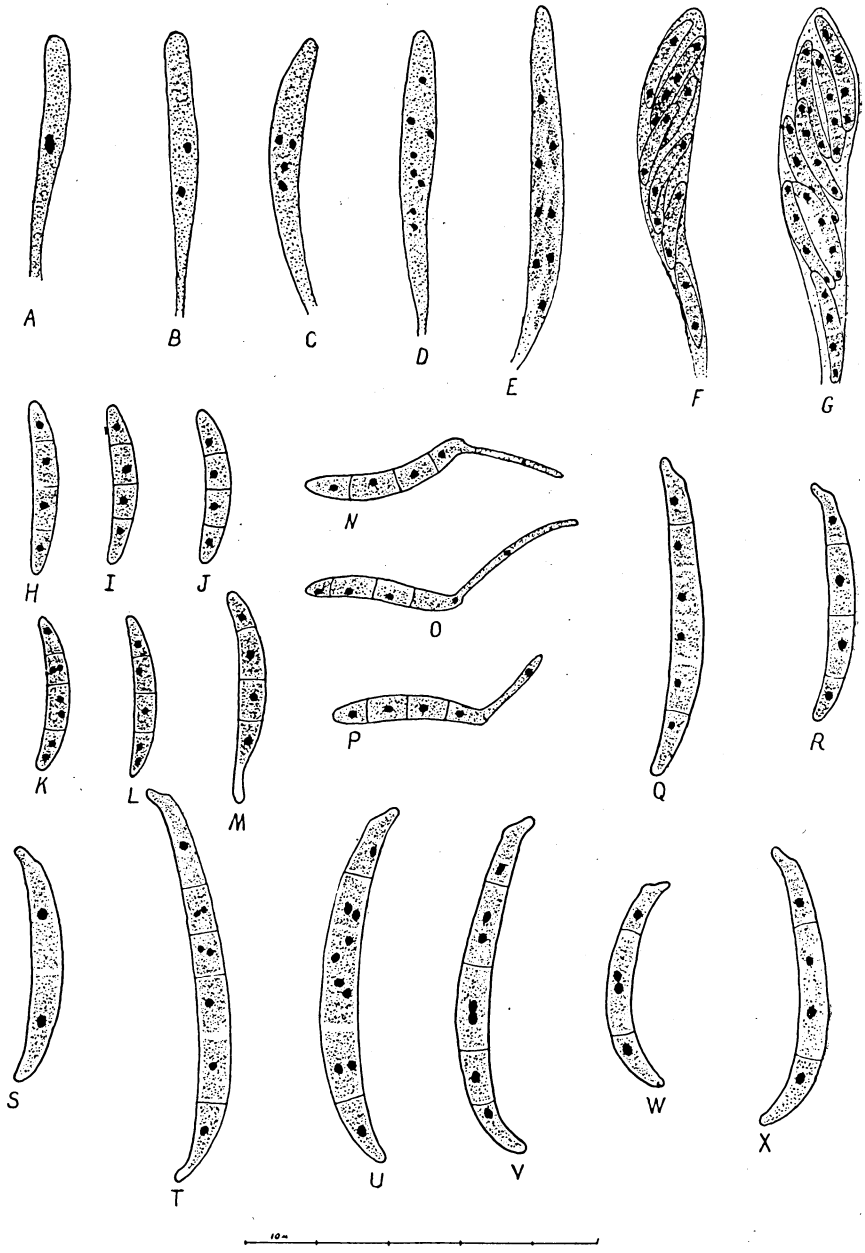

Plate V

Plate V

Ascospore formation and nuclear condition of ascospores and conidia of *Gibberella saubinetii*.

Figs. A, B, C, and D. One, two, four, and eight nucleate stages in the development of the ascus.

Fig. E. Young ascospores being cut out around the nuclei by the process of free cell formation.

Fig. F. Young ascospores in a two-nucleate condition except for one spore which contains four nuclei.

Fig. G. Young ascospores in a four-nucleate condition. Septa are not yet completely formed.

Figs. H, I, J, and M. Mature ascospores, each cell of which contains but one nucleus.

Figs. K and L. Mature ascospores; some cells containing two nuclei.

Figs. N, O, and P. Germinating ascospores.

Figs. Q and R. Mature conidia having but one nucleus per cell.

Figs. S to X, inclusive. Conidia, some of the cells of which contain more than one nucleus.

Drawings made with the aid of a camera lucida. Objective, oil immersion, 100 x; eyepiece, 12 x.

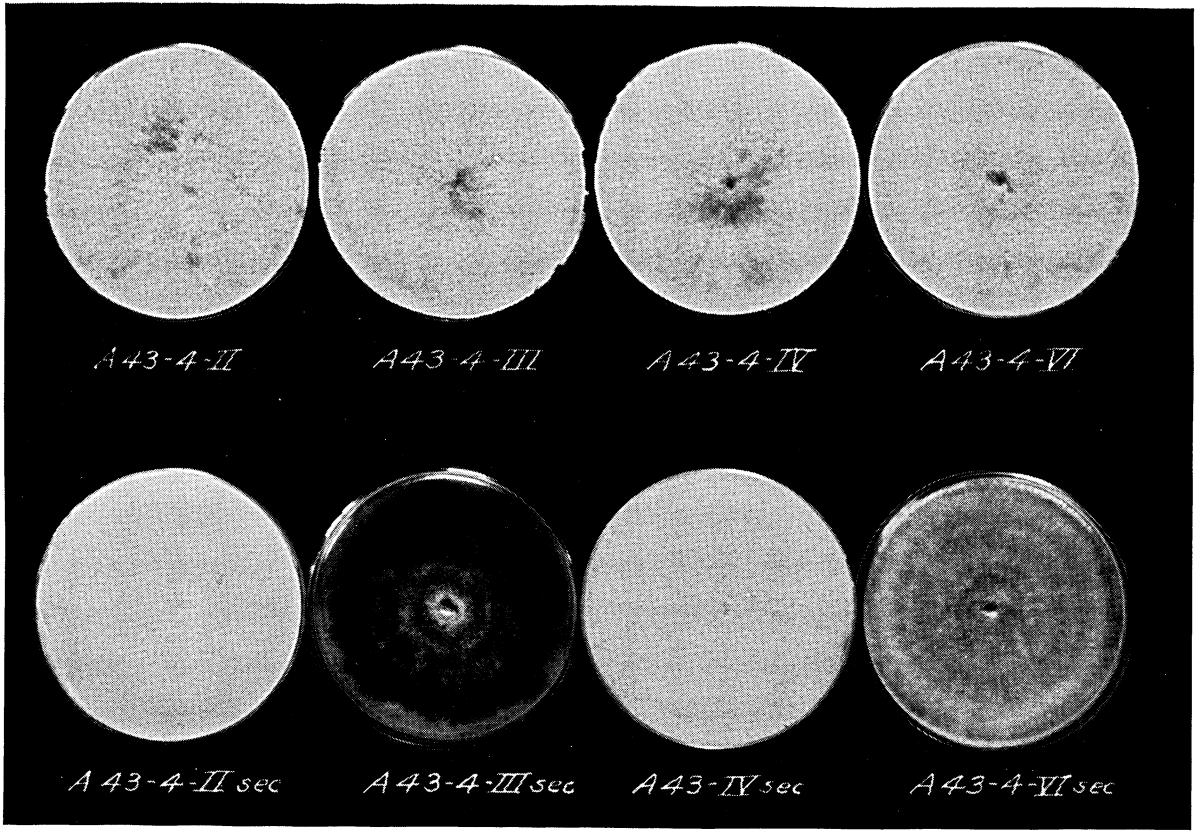

Plate VI

Top view of four single ascospore isolates of *Gibberella saubinetii* and a variant from each. Parents, upper row; variants, lower row.

Plate VII

The relative pathogenicity of four single ascospore isolates of *Gibberella saubinetii* and a variant from each on Prelude wheat.